Vol. 31   June 2006   No. 2

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Effect of fertilizers applied to brinjal on host preference and development of sucking pests

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ABSTRACT: Under screen house conditions, leaf hoppers and whiteflies settled in the organics-applied brinjal plants were comparatively lesser than on plants applied with NPK in inorganic form. Prolonged nymphal duration and low growth index of leaf hopper was recorded in organics applied treatments. No definite trend was observed with nymphal period of whitefly among the different treatments. In FYM + biofertilizers + mahua cake, FYM + biofertilizers, and poultry manure + biofertilizers, treatments less number of whiteflies developed into adults than in the treatment with NPK in inorganic form. Population build up of aphid was less in the organics applied treatments. © 2006 Association for Advancement of Entomology

KEYWORDS: FYM, poultry manure, oil cakes, sucking pests, brinjal

INTRODUCTION

Brinjal or egg plant, *Solanum melongena* L. is one of the most common and extensively grown vegetables all over the country. It is attacked by a number of serious insect pests. The management strategy for these insect pests remains largely confined to pesticides, which led to the endangerment of the ecosystem. It is pertinent that a change in the insect pest management strategy may form a meaningful solution to avoid the ill effects caused by the synthetic chemical insecticides. The mechanism of induced insect resistance in crop plants is gaining the attention of the scientists in recent years. In the absence of natural heritable resistance, resistance can be induced by alternate strategies. Induced resistance is the qualitative and quantitative enhancement of plant's defense mechanism and is a non heritable resistance, where the host plants are induced to impart resistance to tide over pest infestation (Dilawari and Dhaliwal 1993). The present study was therefore designed to evaluate the influence of organic sources of nutrients in changing the host plant nutrition and in turn on the major insect pests of brinjal.

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MATERIALS AND METHODS

Screen house and laboratory experiments were conducted on the influence of the organic sources of nutrients on biological traits of sucking insect pests of brinjal i.e., leaf hopper, *Amrasca devastans* (Dist.), whitefly, *Bemisia tabaci* (Genn.), aphid, *Aphis gossypii* (Glover). Farm Yard Manure (FYM) (12.5 t/ha) and poultry manure (3 t/ha)., with computed quantity were applied basally at the time of main field preparation. The biofertilizers *viz., Azospirillum*, phosphobacteria and silica solubilizing bacteria each @2 kg/ha were incorporated in the soil in the respective treatments. Half of the dose of the total requirements of other organic amendments *viz.,* neem cake, mahua cake and pungam cakes @1000 kg/ha were applied as basal and the remaining half of the dose was applied as top dressing in two equal splits at 20 days interval. Inorganic fertilizers in the form of urea, single super phosphate and muriate of potash with computed quantity were applied at recommended doses (100:50:30). Fifty per cent of total N and entire P and K were applied as basal and the rest of 50 per cent N was applied in two equal splits as top dressing at 20 days interval.

The study on the influence of the organic and inorganic sources of nutrients on feeding preference of leaf hopper and whitefly was carried out as per the method described by Godase and Patel (2001). Fresh uniform sized shoots from the plants grown on various treatments were kept immersed in water in small plastic cups through a hole on the cover of the cups and the hole was plugged with absorbent cotton so as to tightly hold the twigs. These shoots were arranged in a circle at equidistance in insect rearing cages ($45 \times 45 \times 60$ cm). Sixty field-collected adults were released at the centre of the circle. After 60 h, the adults settled on each shoot were recorded.

The study on the influence of the organic and inorganic sources of nutrients on the developmental period of leaf hopper and whitefly was carried out as per the method described by Godase and Patel (2001). For the leaf hopper, 10 newly emerged nymphs were released in each petridish containing fresh leaves kept on filter paper moistened with moist cotton and this treatment setup was replicated thrice. The leaves were changed in alternate days. Third leaf from top was used. For the whitefly, uniform aged and sized shoots in each treatment were selected and covered with perforated butter paper bag, 15 days before initiating the experiment so as to avoid egg laying under natural conditions. Freshly emerged nymphs collected from stock culture along with leaf bit were stapled on selected shoot and again rebagged until whitefly adults emerged.

To study the population buildup of aphid, 45 days old potted plants of each treatment were confined in Mylar film cages separately. In each cage, five adult aphids were introduced. Fifteen days later, total number of aphids developed was counted for each treatment.

TABLE 1. Feeding preference of leaf hopper, *Amrasca devastans* and whitefly, *Bemisia tabaci* infesting brinjal

Treatment	Number of adults observed after 60 h	
	A. devastans	B. tabaci
FYM + Biofertilizers + Neem cake	5 (2.24)[d]	5 (2.24)[a]
FYM + Biofertilizers	3 (1.73)[a]	4 (2.00)[a]
FYM + Biofertilizers + Mahua cake	3 (1.73)[a]	5 (2.24)[a]
Poultry manure + Biofertilizers + Neem cake	5 (2.24)[a]	4 (2.00)[a]
Poultry manure + Biofertilizers	3 (1.73)[a]	4 (2.00)[a]
NPK alone	8 (2.83)[b]	8 (2.83)[b]
Untreated control	4 (2.00)[a]	3 (1.73)[a]

Values in parentheses are square root transformations. In a column, mean followed by same letter are not significantly different at $P = 0.05$ as per DMRT.

RESULTS AND DISCUSSION

Feeding preference

There was significant difference between the adults settled on the inorganic applied treatments and organics applied treatments (Table 1). More number of leaf hoppers and whiteflies were settled on the shoots collected from NPK treated plants. The organic treatments harboured comparatively less population of leaf hoppers and whiteflies. The results indicated that the feeding preference of leaf hopper and whitefly significantly increased with increased levels of nitrogenous fertilization. Similar results of increased whitefly incidence with increased fertilization was reported by Prakash *et al.* (1979) in brinjal, Yein and Harcharan Singh (1982) in green gram and Rote and Puri (1992) in cotton. The increase in the feeding preference of brinjal leaf hopper with increased fertilization is again in agreement with the findings of Godase and Patel (2001). The application of farm yard manure when combined with neem cake and biofertilizers proved to be more effective in reducing the settlement and feeding of hopper and whitefly. No significant difference was observed among the organic treatments (Table 1).

Effect on developmental period

The growth index of leaf hopper and whitefly was significantly more on inorganic fertilizers applied plants than the organic fertilizers applied ones (Table 2). High growth index revealed more survival of nymphs on the plants resulting in normal development of insect. Poultry manure when applied with biofertilizers reduced the growth index considerably. The growth index observed in the treatments with organics was less when compared to NPK in inorganic form. The lower growth index of hopper and whitefly on organics received plants revealed their lower suitability for development. This corroborates the findings of Chandramani (2003). Prolonged nymphal duration in leaf hopper was observed in the treatments where organics alone

TABLE 2. **Effect** of fertilizers applied to brinjal plants on the development of sucking pests

Treatments	*Anrusca devastans*			*Bemisia tabaci*			
	Per cent adult emergence	Average Nymphal duration (Days)	Growth index	Per cent adult emergence	Average Nymphal duration (Days)	Growth index	Number of aphids after fifteen days
FYM + Biofertilizers + Neem cake	73.33 (58.91)^a	11.00 (3.32)^a	6.67 (2.58)^b	63.33 (52.73)^a	10.63 (3.26)^a	5.96 (2.44)^b	29.12 (5.39)^a
FYM + Biofertilizers	70.00 (56.79)^a	10.76 (3.28)^a	6.50 (2.55)^{bc}	60.00 (50.77)^a	10.94 (3.31)^a	5.48 (2.34)^{bcd}	41.00 (6.40)^b
FYM + Biofertilizers + Mahua cake	73.33 (58.91)^a	10.95 (3.31)^a	6.70 (2.59)^b	60.00 (50.77)^a	10.83 (3.29)^a	5.54 (2.35)^{bcd}	35.68 (5.97)^{ab}
Poultry manure + Biofertilizers + Neem cake	70.00 (56.79)^a	11.48 (3.39)^a	6.10 (2.47)^c	63.33 (52.73)^a	10.84 (3.29)^a	5.84 (2.42)^{bc}	36.72 (6.06)^{ab}
Poultry manure + Biofertilizers	70.00 (56.79)^a	11.62 (3.41)^a	6.02 (2.45)^c	60.00 (50.77)^a	11.28 (3.36)^a	5.32 (2.31)^d	43.11 (6.56)^b
NPK alone	76.66 (61.11)^a	10.30 (3.21)^a	7.44 (2.73)^a	66.66 (54.73)^a	10.00 (3.16)^a	6.67 (2.58)^a	80.41 (8.97)^d
Untreated control	70.00 (56.79)^a	11.33 (3.37)^a	6.18 (2.49)^{bc}	60.00 (50.77)^a	11.17 (3.34)^a	5.37 (2.32)^{cd}	64.14 (8.01)^c

Values in parenthesis **are transformed values.** In a column mean followed by same letter are not significantly **different** at $P = 0.05$ as **per** DMRT.

were applied. On the contrary, in NPK applied treatments the leaf hopper development was faster. Similar trend with brinjal leaf hopper at higher levels of nitrogen was observed by Godase and Patel (2001). No definite trend was observed with regard to nymphal duration of whitefly. The high survival per cent of whitefly was recorded in inorganic NPK applied treatments when compared to organics applied plots. The number of leaf hopper and whitefly nymphs developed into adults was more in NPK treated plots than in organics treated. The nymphal survival was less in organic treatments. This is in agreement with the findings of Rossi and Strong (1991) who reported high survivorship of an oligophagous leaf hopper, *Carneocephala floridana* on the most highly fertilized plants. Further, Padhi and Chatterji (1986) reported that the larval survival of rice yellow stem borer was low on resistant varieties TKM 6 and PTB 18 because of their low nitrogen content.

Population buildup of Aphid

Among the various treatments, significant difference was noticed in the population buildup of aphid and it was less in the treatment with FYM + biofertilizers + neem cake followed by the combination of FYM + biofertilizers + mahua cake than the inorganic NPK applied treatments. The corresponding per cent reduction of these treatments over NPK was 63.75 and 56.25 (Table 2). The possible reason for the higher population buildup of aphid on inorganic fertilizers applied plants is that the inorganic forms increases the plant growth and provide the nutrients to the plants in large quantities for shorter period. Thereby the plants are endowed with luxuriant growth which offers adequate food to the insects leading to heavy insect population. This is in corroboration with Godase and Patel (2001) who reported heavy incidence of leaf hoppers and aphids on brinjal with higher levels of nitrogenous fertilization. The organic manures act like slow release fertilizers providing balanced nutrition to plants and facilitates balanced growth, finally making them less prone to pest incidence.

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Conservation of natural enemies through IPM in brinjal (*Solanum melongena* L.) fields

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ABSTRACT: Large-scale IPM farm trials were carried out on brinjal in Raispur village, Ghaziabad, Uttar Pradesh during 2003–04 and 2004–05. Farmers' practices (FP) consisting entirely of pesticidal sprays were also included in the trial. Significantly higher population of coccinellids, predatory spiders and *Chrysoperla* was observed in IPM fields compared to field adopting farmers' practices (FP) during both the years. Coccinellids and predatory spiders were present throughout the crop season starting from September till mid-March; however, *Chrysoperla* appeared from September to mid-December. As a result of IPM components, pesticide use was reduced from 5–6 sprays to only 1–2 sprays and thus led to build up of natural enemies and consequent reduction in pest load. © 2006 Association for Advancement of Entomology

KEYWORDS: IPM, natural enemies, conservation, insecticides

INTRODUCTION

At National Centre for Integrated Pest Management, New Delhi attempts have been made to validate IPM technology in brinjal in a farmers' participatory approach, with a view to reduce the pesticide load in the environment. Its impact on the population of natural enemies and their ability to keep the pest population under reasonable control were studied over two years from 2003 to 2005. The results are presented in this paper.

MATERIALS AND METHODS

Field trials were carried out at farmers' field in village Raispur, Distt. Ghaziabad (U.P.) in two blocks of 2 acres each during 2003–04 and 10 acres each covering 10 farming families during 2004–05. One block involved the common Farmer's practices prevailing in the area to contain the pest problems whereas the other block involved IPM practices. The trials were conducted with hybrid F1-321 and transplanting of

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TABLE 1. Components of IPM and FP adopted in the brinjal fields

IPM	Farmers' practices
Nursery	**Nursery**
Raised seed bed	No interventions
Soil solarisation	
Seed treatment with *T. viride* @4g/kg seed	
Main field	**Main field**
Installation of Delta traps and yellow sticky traps for hopper and whitefly @2/acre	One application of each of following insecticides
Pheromone traps installed @5/acre for *Leucinodes orbonalis*	Imidacloprid 17.8 SL @ 150 ml/ha
Soil application of *neem* cake @250 kg/ha along the plant rows at 25 days after transplanting	Cypermethrin 25 EC @ 200 g a.i./ha
Three sprays of NSKE @5% against leaf hoppers, aphids, mites and white fly	Monocrotophos 40 EC 0.05%
Six releases of egg parasite, *T. brasiliensis* @1.0 lakh/ha for shoot and fruit borer between 42 DAT – 100 DAT	Acetamiprid 20 SP @ 100 g/ha
Collection and destruction of egg masses, larvae and adults of hadda beetle	Triazophos 40 EC @ 0.04%
Clipping of borer damaged shoots and collection & destruction of damaged fruits	
Rouged out little leaf affected plants	
One spray each of imidacloprid and cypermethrin in the crop season	

nursery was done in the first week of August during both the years and all the standard agronomic practices recommended for the region were strictly followed. The details of various IPM components are detailed in Table 1.

Though several kinds of natural enemies were observed in the IPM fields, the number of the following three, which were present in high numbers and were of relatively sedentary nature were recorded – eggs of *Chrysoperla*; adults of predatory spiders; and grubs, pupae and adults of *Chilomenes* sp. and *Coccinella* sp. Counts were made at weekly intervals on 25 plants/acre in IPM as well as FP fields. Grubs, pupae and adults of *Coccinella* sp. and *Chilomenes* sp. were recorded together as it was difficult to differentiate between larvae and pupae of these two predators. Similarly, the different kinds of spiders present in the field were recorded together as a group. In addition, qualitative observations were made on other predators and parasitoids.
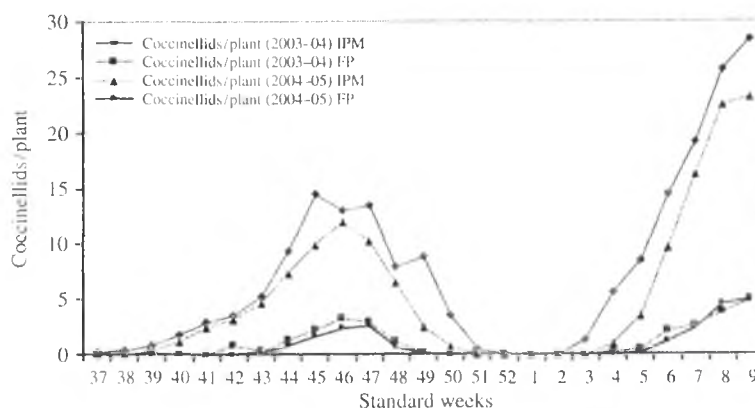
FIGURE 1. Population of coccinellids in brinjal fields.

RESULTS AND DISCUSSION

Common natural enemies i.e. predators and parasitoids observed in the brinjal field are presented in Table 2. The population of several of these natural enemies was low in general and some like *Phenerotoma* sp were observed in traces. Predatory wasps and dragon fly population was not recorded because of their non-sedentary nature. The population of coccinellids, predatory spiders and *Chrysoperla* was recorded during both the years and the data are presented in Table 3. The results indicated that coccinellids started appearing early in the season i.e. mid-September and its population declined during Dec-Jan and it reappeared in Feb-March. Peak population (14.47, 11.94, 28.42 and 23.32) was observed during 45th–47th and 9th week during both the years. Coccinellid population declined during Dec-Jan probably due to hibermation of host species couped with low temperature. The mean population (6.74/plant) remained significantly higher in IPM fields are compared to FP (0.97/plant) through out the crop seasons. During 2003–04 season, coccinellids appeared relatively early in the crop and were relatively higher as against 2004–05 season. This was probably due to seasonal/crop variation and only one application of chemical insecticide during 2003–04 and two applications during 2004–05 (Fig. 1).

The population of the predatory spiders was slightly higher in IPM field as compared to FP fields and a similar trend was observed during both the years. Peak of 4.69 and 3.24 spiders per plant was observed in 46th week in IPM fields during both the years, while it was lower, being 2.27 and 1.02 per plant in 9th week i.e. March in FP fields during the years (Fig. 2).

Chrysoperla eggs were observed from the 36th standard week i.e. first week of September onwards during 2003–04 and 2004–05 and remained active up to mid-December (50th standard week). Population of *Chrysoperla* eggs was significantly higher in IPM fields than the FP fields and peak of 4.15 and 3.02 eggs per plant in IPM and 0.64 and 0.32 eggs per plant in FP was observed during 2003–04 and

TABLE 2. Important natural enemies of insect pests of brinjal encountered in IPM and FP fields

Scientific name	Common name	Host insect	Relative abundance	
			IPM	FP
Menochilus sexmaculatus	Lady bird beetle	Leaf hoppers (nymphs & adults)	Very high	Very low
Coccinella septumpunctata	Lady bird beetle	Leaf hopers (nymphs & adults)	Very high	Very low
Chrysoperla carnea	Lace wing bug	Leaf hoppers, whiteflies & mites	Medium	Very low
Crocothemis servilia	Dragon fly	Leaf hopper nymphs, adults, borer moths	High	Medium
Ischnura sp	Damsel fly	Leaf hopper nymphs, adults, borer moths	High	Medium
Mantis religiosa	Praying mantis	Leaf hopper nymphs, adults, borer moths	Medium	Very low
Cyrtorrhinus lividipennis	Mirid bug	Leaf hopper nymphs & adults	Very low	Nil
Trathala sp	Ichneumonid	Larvae of shoot & fruit borer	Very low	Traces
Dicrurus adsimilis	Drongo (Bird)	Larvae	Very low	Very low
Erioborus argenteopilous	–	Larvae	Very low	Very low
Bracon sp	–	Larvae	Very low	Traces
Phenerotoma sp	–	Larvae	Very low	Traces
Lycosa pseudoannulata	Wolf spider	Border moths, leaf hopper nymphs	Medium	Low
Atypena (Callitrichra) Formosa	Dwarf spiders	Leaf hopper nymphs	Medium	Low
Argiope catenulate	Orb spider	Leaf hopper nymphs	Medium	Low
Tetragnatha maxillosa	Long jawed spider	Leaf hopper nymphs, flies & moths	Medium	Low
Prestes sp	Predatory wasp	Larvae, leaf hopper nymphs, flies	Low	Very low

2004–05, respectively (Fig. 3). It was also seen that there was heavy build up of aphid population in Feb–March but *Chrysoperla* did not reappear after winter which could probably be due to competition and dominance of Coccinellids. Earlier a large build up of natural enemies, especially *Chrysoperla* sp. and predatory spiders, was reported in cotton IPM fields by Tanwar *et al.* (2005) where neem, bioagents like *Trichogramma* and pheromone traps were employed. The population of these predators was negligible in FP fields where several rounds of pesticides sprays and their mixtures were applied by the farmers.

It is evident from the above results that with the application of neem products, release of bio-agents and periodical rouging out and destruction of infested twigs and

TABLE 3. Mean natural enemies population in IPM and Farmer's practice (FP) brinjal fields at Raispur, Ghaziabad

Treatments	Coccinellids/plant		Mean	Predatory spiders/plant		Mean	Chrysoperla eggs/plant		Mean
	2003–04	2004–05		2003–04	2004–05		2003–04	2004–05	
IPM	7.54	5.95	6.74	2.03	1.50	1.76	1.69	1.55	1.62
FP	1.05	0.89	0.97	0.35	0.18	0.26	0.09	0.064	0.077
't' value	4.891*	4.359*		6.973*	7.006*		4.978*	4.962*	

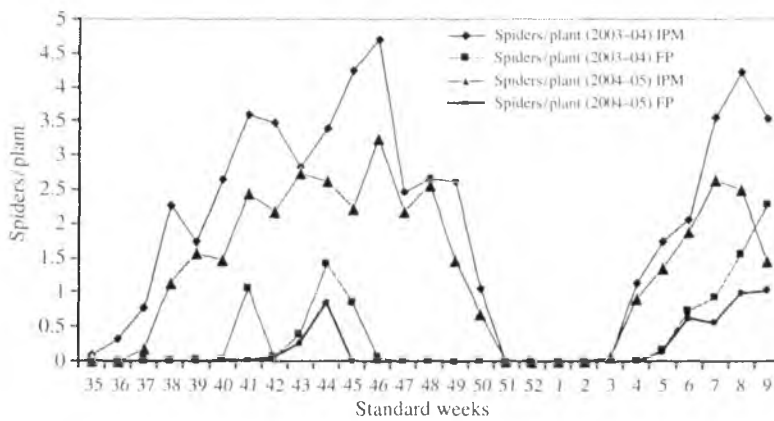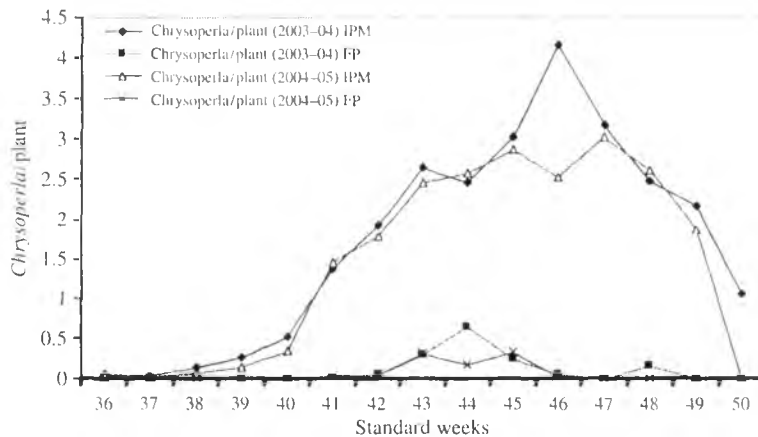*Paired 't' test significant at p - 5%.

FIGURE 2. Population of predatory spiders in brinjal fields.

FIGURE 3. Population of *Chrysoperla* in brinjal fields.

fruits, the application of chemical pesticides can be reduced considerably and at the same time population of natural enemies can also be enhanced to a greater extent in vegetable based ecosystems where natural enemies have been severely affected by the repeated large scale and exclusive use of chemical pesticides. Maleque *et al.* (1999) reported that ladybird beetles and spiders were seriously affected in the brinjal crop where Cymbush 10EC (cypermethrin) was applied at weekly intervals compared with fields where mechanical control and few sprays were applied, and unsprayed fields. Hull and Beers (1985) reported the use of selective pesticides as the most powerful means of integrating pesticide use and natural enemies.

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Cry diversity of *Bacillus thuringiensis* isolates of Western Ghat region and their bioefficacy against *Spodoptera litura* and *Helicoverpa armigera*

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ABSTRACT: Bioefficacy of crude protein of 15 *B. thuringiensis* strains isolated from Western Ghat region was examined against second instar larvae of *S. litura* and *H. armigera*. Among the various strains tested, SW 25 was found most effective against both the insect larvae (LC_{50} of 966.08 and 775.06 ppm and LT_{50} of 55.60 and 52.11 hours, respectively) and had overlapping fiducial limits with that of the reference strain, HD1. Among the ten best performed isolates subjected to amplification of the specific *cry* genes *viz.*, *cry1Ac* (3.5 kb), *cry3Aa* (2.24 kb) and *cry10Aa* (2.37 kb), *cry1Ac* was found to be amplified in the strains SW 25, DKS 2(3), SWPB, SW 15, DKS 8(2) and SWPT. While the primers specific for *cry 3Aa* could amplify the gene in DKS 2(3) and DKS 4(1), the primers specific for *cry10Aa* could not amplify the gene in any of the strains including the reference HD1 and D1.
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INTRODUCTION

Among microbial biocontrol agents of insect pests, *B. thuringiensis* is reported to be the most successful commercially. Its biocontrol activity is due to the insecticidal crystal protein (ICP) or delta endotoxin produced in the sporulating cells (Nagamatsu *et al.*, 1998). The ICPs (Cry proteins) of different strains of *B. thuringiensis* vary in their toxicity against different insects (Shin *et al.*, 1995).

Most of the genes coding for ICP appear to reside in plasmids (Gonzalez *et al.*, 1981). More than 200 *cry* genes have been sequenced upto the year 2002 and classified into 40 groups and different subgroups based on their amino acid similarity (Crickmore *et al.*, 2002). However, gaining of resistance to cry proteins by the pests is the greatest threat to the successful use of *B. thuringiensis* based formulations and

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transgenic crops (Hokkan and Wearing, 1995). A significant number of pests are not controlled with the available Cry proteins (Johnson and Bishop, 1996) making it essential to search for new isolates with novel *cry* genes. Western Ghats is one of the 18-biodiversity hot spots in the world, and is least explored for microbial diversity. In this paper, we report the *cry* diversity of *Bacillus thuringiensis* strains of Western Ghat region and their bioefficacy against *Spodoptera litura* and *Helicoverpa armigera*.

MATERIAL AND METHODS

Fifteen *B. thuringiensis* strains isolated from the Western Ghat region of Uttar Kannada district of Karnataka and Palakkad district of Kerala, along with two reference strains (HD1 and D1 obtained from the Department of Biotechnology, University of Agricultural Sciences, Dharwad) were used in the present study.

Bioefficacy of crude protein of *B. thuringiensis* isolates against *S. litura* and *H. armigera*

All the 15 isolates and the reference strain, HD1, were grown in Modified Glucose Medium (Travers *et al.*, 1987) for 72 h. The crude protein extraction and protein estimation were done by standard methodologies (Dulmage, 1970, Plummer, 1979). The bioefficacy of crude proteins of *B. thuringiensis* isolates was tested at different concentrations against second instar larvae of *S. litura* and *H. armigera*.

Six different concentrations of crude protein (5, 50, 500, 1000, 2500 and 5000 ppm) of each isolate including reference strain HD1 were prepared by dissolving the calculated amount of spore crystal complex in sterile distilled water. Fresh leaf discs of 35 cm^2 area were dipped in different concentrations of crude protein, air dried and fed to the pre-starved (for 6 h) second instar larvae. Castor leaf was used for *S. litura* and chickpea leaf for *H. armigera*. Suitable untreated control was also maintained where the leaf discs dipped in sterile distilled water were fed to the larvae. The experiment was conducted by treating 30 larvae for each treatment. *S. litura* larvae were maintained in plastic petriplates and *H. armigera* larvae in individual vials. The larvae in all the treatments were fed with normal leaves on subsequent days upto five days. The observation on larval mortality was recorded at an interval of 24 h for five days.

Molecular diversity analysis of the isolates

Ten selected efficient isolates [SW 25, DKS 2(3), SWPB, DKS 4(1), SW 15, DKS 8(2), YSP 3, SWPT, SW 22, DKS 5(1)] along with two reference strains (HD1 and D1) of *B. thuringiensis* were subjected to molecular diversity analysis and characterization by specific PCR.

The total DNA was isolated from the selected *B. thuringiensis* isolates and reference strains by following the protocol of Sambrook and Russel (2001).

Three *cry* specific primers were used for the amplification of the full-length genes *viz.*, *cry1Ac* (3.5 kb), *cry3Aa* (2.24 kb) and *cry10Aa* (2.37 kb). The primers for

cry3Aa and *cry10Aa* were designed using gene tool software and custom synthesized from Bioserve Biotechnologies (India) Pvt. Ltd., Hyderabad, while the primer for *cry1Ac* was synthesized at Bangalore Genei, Bangalore. The different primers used for amplification of the specific *cry* genes were as follows:

Gene recognized	Size of band expected to be amplified	Sequence	Reference
Cry1Ac	3.5kb	F: 5' TGGAGGATCCATATGGTAACAATCCGAACA 3' R: 5' AGTTGGATCCTTACTATTCCTCCATAAGG 3'	Kumaraswamy (2005)
Cry3Aa	2.25kb	F: 5' GGATGCCTAAAAACGAAGAACA 3' R: 5' TTTCCTACTTTCATTGTTCCCTTA 3'	Present study
Cry10Aa	2.37kb	F: 5' TGTCGGAAAATGATGAGGTTA 3' R: 5' GCACCACTTGTAAACATAGGG 3'	Present study

The Taq DNA polymerase, 10x assay buffer and individual dNTPs were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore and the PCR was carried out by using standard procedure (Sambrook and Russel, 2001) with the help of an eppendorf model thermal cycler with the following temperatures. Initial denaturation at 94 °C for 5 min (1 cycle) followed by 44 cycles (each at 94 °C for 2 min for denaturation, 45 °C for 1 min for annealing and 72 °C for 2 min for extension) and a final extension step of 72 °C for 30 min (1 cycle). PCR products were resolved on a one percent agarose gel stained with ethidium bromide and loaded with λ DNA Hind III digest as DNA molecular weight marker. The DNA bands in gels were documented using gel documentation system (Uvitec Cambridge, England).

RESULTS

The dose mortality response of second instar larvae of *S. litura* to crude protein of *B. thuringiensis* isolates revealed that the isolate SW 25 was most efficient among the isolates with an LC_{50} value of 966.08 ppm (fiducial limits ranging from 654.90–1269.20 ppm) followed by DKS 2(3), SWPB, DKS 4(1) and YSP 3 with LC_{50} values 1141.84, 1217.73, 1362.75, 1498.10 ppm, respectively and were found to have an overlapping fiducial limits with the reference strain HD1 which showed an LC_{50} value of 876.41 ppm with fiducial limits ranging from 633.00 to 1121.20 ppm (Table 1).

The time mortality response studies against second instar larvae of *S. litura* at a crude protein concentration of 2500 ppm up to 120 h at an interval of 24 h revealed that SW 25, among the isolates, possessed the least LT_{50} value of 55.60 h with fiducial limits ranging from 45.95 to 64.76 h (Table 1). The other three isolates, viz., DKS 2(3), SWPB and DKS 4(1), also possessed overlapping fiducial limits with the reference strain, HD1 (LT_{50} of 49.54 with fiducial limits, 41.33–57.04 h) and showed LT_{50} values 61.28, 66.24 and 65.21 h respectively.

The dose mortality response of second instar larvae of *H. armigera* to crude protein of *B. thuringiensis* isolates revealed that the isolate SW 25 showed the least LC_{50} of 775.06 ppm with fiducial limits ranging from 402.50 to 1048.50 ppm (Table 2). The isolates DKS 2(3), DKS 4(1), DKS 8(2), SW 15 and SWPB showed the LC_{50} values of 855.42, 979.52, 1245.82, 1118.82 and 901.97 ppm, respectively, with an overlapping fiducial limits with the reference strain (HD1).

TABLE 1. Dosage and time mortality response of *S. litura* to crude protein of *B. thuringiensis* strains

| Isolate | Dosage mortality | | | | | Time mortality | | | | |
| | χ^2 | LC_{50} (ppm) | Fiducial limits (ppm) | | $b \pm$ S.E. | χ^2 | LT_{50} (hours) | Fiducial limits (hours) | | $b \pm$ S.E. |
			Lower	Upper				Lower	Upper	
DKS 2(3)	8.70	1141.84[ab]	825.3	1485.2	2.52 ± 0.47	7.53	61.28[ab]	50.33	72.46	3.24 ± 0.56
DKS 4(1)	3.73	1362.75[ab]	952.7	1834.4	2.15 ± 0.43	6.97	65.21[ab]	53.60	77.84	3.10 ± 0.56
DKS 5(1)	3.82	2159.17[bc]	1574.2	3036.0	2.14 ± 0.43	6.02	87.86[bc]	70.97	119.22	2.50 ± 0.57
DKS 8(1)	4.70	2910.04[bc]	2176.4	4933.1	2.63 ± 0.61	4.59	108.34[bc]	84.70	178.39	2.30 ± 0.59
DKS 8(2)	0.57	2289.6[bc]	1618.6	3454.9	1.87 ± 0.41	5.29	95.83[bc]	76.68	138.49	2.43 ± 0.58
DKS 10(2)	4.30	2849.28[bc]	2065.3	4343.3	2.07 ± 0.45	4.20	110.59[bc]	86.25	185.57	2.31 ± 0.60
SW 15	2.82	1607.68[b]	1152.1	2180.5	2.15 ± 0.42	6.75	73.43[bc]	60.06	90.81	2.79 ± 0.56
SW 18	4.45	2649.8[bc]	1927.5	3934.1	2.09 ± 0.44	5.47	104.32[bc]	81.69	167.38	2.26 ± 0.58
SW 22	4.51	1777.43[bc]	1269.9	2468.0	2.05 ± 0.41	6.50	76.14[bc]	62.31	95.17	2.76 ± 0.56
SW 25	7.59	966.08[ab]	654.9	1269.2	2.42 ± 0.50	7.30	55.6[ab]	45.95	64.76	3.67 ± 0.58
SWPB	7.39	1217.73[ab]	895.3	1579.0	2.57 ± 0.46	7.21	66.24[ab]	54.35	79.47	3.03 ± 0.56
SWPT	1.38	1948.67[bc]	1426.7	2671.0	2.21 ± 0.43	7.33	79.48[bc]	64.49	102.38	2.59 ± 0.55
YSP 3	2.78	1498.1[ab]	1019.2	2074.4	1.96 ± 0.43	6.28	72.05[bc]	59.31	87.77	2.94 ± 0.57
YSP 5(1)	4.23	3182.06[c]	2291.6	5101.8	2.06 ± 0.47	5.16	113.03[c]	86.90	201.33	2.19 ± 0.58
YSP 6	4.51	3422.1[c]	2457.2	5693.5	2.07 ± 0.50	4.74	119.71[c]	90.61	233.64	2.14 ± 0.59
HD1	8.99	876.41[a]	633.0	1121.2	2.83 ± 0.54	7.26	49.54[a]	41.33	57.04	4.34 ± 0.63

HD 1: Reference strain; Total no. of concentrations of crude proteins used: 6; df for LC_{50}: 4; Total no. of time intervals used: 5; df for LT_{50}: 3

TABLE 2. Dosage and time mortality response of *H. armigera* to crude protein of *B. thuringiensis* strains

Isolate	Dosage mortality					Time mortality				
	χ^2	LC_{50} (ppm)	Fiducial limits (ppm) Lower	Upper	b ± S.E.	χ^2	LT_{50} (hours)	Fiducial limits (hours) Lower	Upper	b ± S.E.
DKS 2(3)	6.48	855.42[ab]	570.8	1119.4	2.52 ± 0.53	6.49	53.65[ab]	44.63	62.05	3.98 ± 0.61
DKS 4(1)	5.46	979.52[ab]	700.5	1269.5	2.59 ± 0.48	7.08	55.8[ab]	46.28	64.81	3.76 ± 0.59
DKS 5(1)	4.07	1720.52[b]	1250.7	2333.2	2.19 ± 0.41	6.58	70.82[b]	58.14	86.25	2.92 ± 0.56
DKS 8(1)	1.05	1895.32[b]	1381.4	2606.5	2.15 ± 0.41	7.19	70.89[b]	58.03	86.66	2.86 ± 0.56
DKS 8(2)	2.65	1245.82[ab]	757.1	1739.8	1.84 ± 0.45	6.95	58.8[ab]	48.55	68.86	3.48 ± 0.58
DKS 10(2)	4.37	2018.93[b]	1480.2	2782.0	2.20 ± 0.42	6.50	76.14[b]	62.31	95.17	2.76 ± 0.56
SW 15	3.06	1118.82[ab]	747.4	1501.1	2.16 ± 0.45	7.49	57.21[ab]	47.27	66.78	3.58 ± 0.58
SW 18	4.61	2347.04[b]	1745.7	3229.3	2.36 ± 0.46	6.55	82.4[b]	67.03	107.18	2.61 ± 0.56
SW 22	1.47	1665.15[b]	1196.2	2271.1	2.12 ± 0.41	6.89	67.38[b]	55.44	80.85	3.05 ± 0.56
SW 25	7.02	775.06[a]	402.5	1048.5	2.30 ± 0.61	7.36	52.11[ab]	43.28	60.28	4.02 ± 0.61
SWPB	7.44	901.97[ab]	589.1	1190.0	2.40 ± 0.52	7.21	54.94[ab]	45.5	63.83	3.77 ± 0.59
SWPT	4.96	1559.78[b]	1098.5	2139.3	2.04 ± 0.40	7.57	64.18[b]	52.67	76.51	3.11 ± 0.56
YSP 3	2.62	1401.98[b]	944.9	1926.0	1.99 ± 0.43	6.95	64.23[b]	52.88	76.32	3.18 ± 0.57
YSP 5(1)	4.38	2195.91[b]	1637.4	2981.7	2.39 ± 0.45	7.60	76.48[b]	62.06	97.08	2.62 ± 0.55
YSP 6	4.85	2465.95[b]	1856.3	3361.5	2.48 ± 0.48	5.77	87.31[b]	70.94	116.59	2.58 ± 0.57
HD 1	8.61	715.9[a]	522.5	900.3	3.26 ± 0.68	6.58	44.73[a]	38.76	49.89	7.76 ± 1.30

HD 1: Reference strain; Total no. of concentrations of crude proteins used: 6; df for LC_{50}: 4; Total no. of time intervals used: 5; df for LT_{50}: 3

TABLE 3. The *cry* gene profile of the *B. thuringiensis* isolates

Strains	CrylAc	Cry 3Aa	Cry 10Aa
HD1	+	–	–
D1	+	+	–
SW 25	+	–	–
DKS 2(3)	+	+	–
SWPB	+	–	–
DKS 4(1)	–	+	–
SW 15	+	–	–
DKS 8(2)	+	–	–
YSP 3	–	–	–
SWPT	+	–	–
SW 22	–	–	–
DKS 5(1)	–	–	–

HD1 and D1 are Reference strains.

The time mortality response of second instar larvae of *H. armigera* at the crude protein concentration of 2500 ppm up to 120 h at an interval of 24 h revealed that the isolate SW 25 was having the least LT_{50} value of 52.11 h with fiducial limits ranging between 43.28 and 60.28 h (Table 2). The other five isolates *viz.,* DKS 2(3), DKS 4(1), DKS 8(2), SW 15 and SWPB showed LT_{50} values of 53.65, 55.8, 58.8, 57.21 and 54.94 h respectively, and were found to have an overlapping fiducial limits with the reference strain, HD1 (LT_{50} 44.73 h with fiducial limits, 38.76–49.89 h).

The *cry* diversity analysis of the ten selected isolates and two reference strains (HD1 and D1) using the specific primers *cryIAc, cry3Aa* and *cry10Aa* revealed amplification of *cryIAc* in SW 25, DKS 2(3), SWPB, SW 15, DKS 8(2) and SWPT (Table 3 and Fig. 1). The two reference strains, HD1 and D1, also amplified the *cryIAc* gene. Primers specific for *cry3Aa* could amplify the gene in DKS 2(3) and DKS 4(1) (Table 3 and Fig. 1). The reference strain D1 was also found to contain the gene *cry 3Aa* but all the other strains including the reference strain HD1 could not amplify the gene. The primer specific for *cry10Aa* could not amplify the gene in any of the isolates including the reference HD1 and D1 (Table 3 and Fig. 2).

DISCUSSION

The mortality of second instar larvae was found to increase in general with increase in concentration of crude protein. The dose mortality response of *S. litura* to the crude protein of *B. thuringiensis* isolates indicated a high variability among the isolates to kill the insects. SW 25, among the isolates, showed the least LC_{50} value followed by DKS 2(3), SWPB, DKS 4(1) and YSP 3, and were having overlapping fiducial limits with that of reference strain HD1. The crude protein from other isolates, however showed much less insecticidal activity. Similar differences in effectiveness among strains and subspecies of *B. thuringiensis* against *S. litura* and other insect pests have
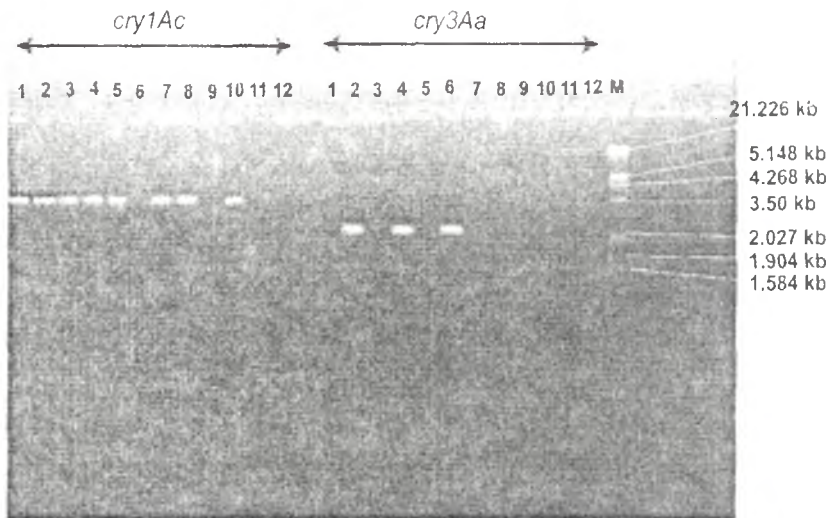
FIGURE 1. *cry1Ac* and *cry3Aa* profile of native *B. thuringiensis* isolates
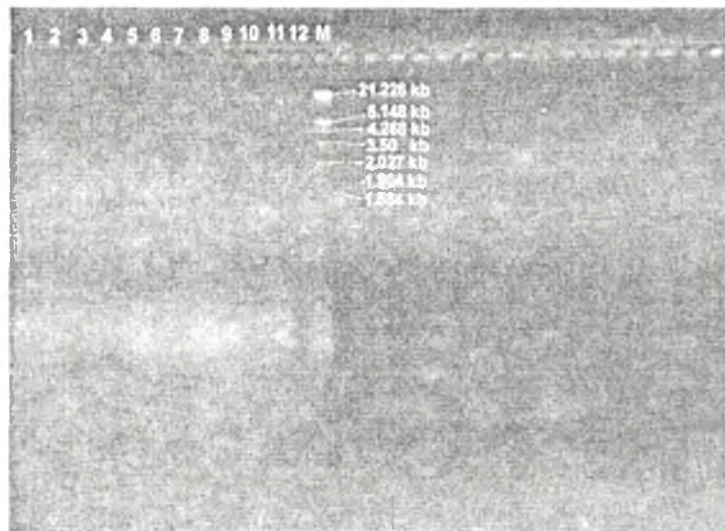

FIGURE 2. *cry10Aa* profile of native *B. thuringiensis* isolates.
(Note: The serial numbers of the wells in gel photographs of Figures 1 & 2 correspond to the serial numbers of isolates in Table 3; M refers to Molecular weight marker.

been reported earlier (Chich Yeong, 1990; Zaz, 1990; Zhong *et al.*, 2000). Variations in the effectiveness among different concentrations of spore or crystal toxins of *B. thuringiensis* are also known (Ahmed *et al.*, 1988; Zaz, 1989).

It was clear from the time mortality response that, irrespective of the isolates, the maximum mortality of *S. litura* was caused on the second day. Among the isolates, SW 25 had the quickest knock down action followed by DKS 2(3), DKS 4(1), and SWPB, which had overlapping fiducial limits with the reference strain HD1. Prabagaran *et al.* (2002) also observed similar time mortality response with native *B. thuringiensis* isolates against *S. litura* when applied at a concentration of 2 $\mu g/cm^2$ spore-crystal complex.

The bioefficacy of crude protein of the isolates against *H. armigera* was almost similar to that of *S. litura*. SW 25, among the isolates, showed the least LC_{50} value followed by DKS 2(3), SWPB, DKS 4(1), SW 15 and DKS 8(2) and had overlapping fiducial limits with that of the reference strain, HD1. The dose mortality response showed a great variability among the isolates to kill the insect. Such differences in the insecticidal activity of different isolates of *B. thuringiensis* may be ascribed to the differences in the carbohydrate affinity of the domain II of the Cry proteins resulting in variable binding specificity with the receptors at the brush border membrane of the insect larvae leading to differences in toxicity of the Cry protein (Smedly and Ellar, 1996). It was observed in the present investigation that the isolates in general were more effective against *Helicoverpa* than against *Spodoptera*. Martinez *et al.* (2004) also observed differential effectiveness of purified crude protein of *B. thuringiensis aizawai* (strain HV 4-2) against *Helicoverpa* and *Spodoptera* but the purified crude protein of HV 4-2 was more effective against *Spodoptera* than against *Helicoverpa*.

The time mortality response of the isolates against *H. armigera* revealed that the isolate SW 25 had the fastest knockdown action followed by DKS 2(3), SWPB, DKS 4(1), SW 15 and DKS 8(2) and had overlapping fiducial limits with the reference strain HD1. Among the isolates, SW 25 and DKS 2(3) also showed quick knockdown action against *Spodoptera* and possessed the least LC_{50} values against both the insect pests. The quick killing action of these isolates might be due to the activity of the specific crystal proteins, which facilitate the synergistic effect of the spores. It is reported that the spores contaminate the hemocoel through a channel created in the midgut membrane and multiply at faster rate utilizing the nutrients from haemolymph (Ali *et al.*, 1985; Wilson and Benoit, 1990; Borgonie *et al.*, 1995), which might lead to bacterial septicemia and death of the larvae within 2–3 days of ingestion (Ali *et al.*, 1985).

The strains of *B. thuringiensis* show wide range of specificity for the insects of different orders and are governed by different classes of *cry* genes present in the strains (Bravo *et al.*, 1998). Analysis of the potential of the strains against different orders of insects by bioassay is an exhaustive but time-consuming process. Of the alternative methods available, PCR analysis is considered to be a good choice, as it allows rapid determination of the specific *cry* genes, high sensitivity and easy adaptability (Ceron *et al.*, 1995). The PCR amplification carried out with 10 isolates using three pairs of specific primers indicated the presence of *cry1Ac* (3.5 kb) in isolates SW 25, DKS 2(3), SWPB, SW 15, DKS 8(2) and SWPT as well as in reference strains, HD1 and D1. However, the remaining four isolates did not amplify *cry1Ac* gene indicating that

their biocontrol activity against Lepidopteran insects could be due to the presence of some other genes specific against lepidoptera. The other lepidopteran specific *cry* genes include *cry1Aa, cry1Ab, cry1B, cry1C, cry1D, cry2A, cry2B* (Federici, 1999).

The isolates DKS 2(3) and DKS 4(1), and the reference strain D1 showed amplification of *cry3Aa* gene indicating their potential against coleopteran insects. The remaining eight isolates and the reference strain, HD1, could not amplify *cry3Aa* gene, indicating the absence of *cry3Aa* gene in these isolates. None of the isolates including the two reference strains could amplify the gene *cry10Aa*, specific against dipteran insects, revealing the absence of *cry10Aa* in these isolates. From the present study, amplicons from PCR of *cry1Ac* and *cry3Aa* specific primers were obtained. These can be cloned into a suitable expression vector to understand the potency of the genes and their further exploitation.

ACKNOWLEDGEMENT

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Effects of juvenile hormone analogue and ecdysone agonist on the spermatogenesis in *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae): a flow-cytometric study

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ABSTRACT: Spermatogenesis of *Spodoptera mauritia* has been studied by employing flow-cytometry. Temporal changes in the frequency of 1C (haploid), 2C (diploid) and 4C (tetraploid) cells were observed from day 0 to day 4 of the last instar larva. Prespermiogenic processes of spermatogenesis occur continuously in the last instar larva with 1C cells appearing in day 0. The thorax-ligated larvae (which lacked retrocerebral endocrines and prothoracic glands) treated with juvenile hormone analogue hydroprene showed no effect on mitotic division of gonial cells but inhibited meiotic divisions. In the testes of the thorax-ligated larvae treated with the ecdysone agonist RH 5992, the proportion of 2C and 4C cells is very high which suggest that the ecdysone agonist accelerates the cell cycling process.
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KEYWORDS: *Spodoptera mauritia*, spermatogenesis, flow-cytometry

INTRODUCTION

Although a considerable information exists on the histological and ultrastructural aspects of spermatogenesis in Lepidoptera, a quantitative approach to the temporal pattern of germ cell development is lacking. Flow-cytometry has been successfully employed in other insect species to study spermatogenesis (Yaginuma *et al.*, 1988; Sherwood *et al.*, 1989) and to analyse other physiological processes in insects (Wolbert and Kubbies, 1983; Grimes and Happ, 1987). In the present study, the flow-cytometric technique has been utilized to analyse the temporal relationship between organismal and male germ cell development occurring in the last instar larva of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). Also some experiments were conducted to explore the effects of a juvenile hormone analogue (JHA) and non-steroidal ecdysone

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agonist, RH 5992 on the cell cycle mechanism of spermatogenesis in the last instar larva of *S. mauritia*.

MATERIALS AND METHODS

Insects

The sixth instar (last instar) larvae of *Spodoptera mauritia* of various age groups were obtained from the laboratory stock culture, reared and maintained as described earlier (Nair, 1981). The age of the larvae was designated as day n where day 0 indicates the day of ecdysis to this stage. In the laboratory conditions the last instar larvae had a larval period of 6 ± 0 days.

Cell preparation

The testes from twenty larvae were used for preparation of cell suspension.

The testes were dissected in cold insect saline. They were cleaned off the fat body, tracheae and other tissues and quickly minced with a sterile razor blade to release the contents into cold-buffered saline in a petridish. The cell suspension was vigorously aspirated through 18 ga syringe several times to separate the cells. The cells were filtered through a nylon mesh and centrifuged at 2000 rpm for three minutes. The cells were resuspended in chilled saline and centrifuged at 2000 rpm and the supernatant was discarded. The cells were fixed very slowly in chilled ethanol containing 30% insect saline (140 mM NaCl, 10 mM KCL, 7 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Trehalose, 4 mM $NaHCO_3$, 5 mM TES and 100 mM sucrose) at pH 7.0. The fixed cells were stored at 4 °C until analysis. Prior to analysis, aliquots of cells were washed immediately in phosphate buffered saline (PBS) and passed through 18 ga syringe a few times. The cells were pelleted by centrifugation at 3000 rpm for 5 min and the supernatant was discarded. The cells were resuspended in propidium iodide (50 μg/ml) in PBS supplemented with RNAase (10 μg/ml) and kept in ice overnight to stain the cellular DNA.

Flow-cytometry

Flow-cytometry was performed on a Becton Dickinson FACSCAN and analysed on a computer system. Cellular DNA was stained with propidium iodide, excited at 488 nm and the emitted fluorescence measured above 610 nm. Individual cells were analyzed on the basis of DNA content and size. The histograms of the frequency of different classes of cells i.e., 1C (haploid), 2C (diploid) and 4C (tetraploid) at different time intervals were collected. Cell doublets and cellular debris were eliminated from analysis by electronic gating of collected histograms. Ten thousand cells were utilized for analysis of each sample. The relative position of peaks corresponding to different cellular DNA contents was first established by analysis of the cells from the sixth instar day 1 larva which contains 1C, 2C and 4C cells in the testis.

Ligation and hormone treatment

The sixth instar day 1 larvae were anaesthetized using diethyl ether and tightly ligated between pro - and mesothorax using a silk thread. These larvae were designated as 'thorax ligated', which lack not only brain with retrocerbral endocrine complex but also the prothoracic glands.

The JHA (hydroprene, ethyl 3,7,11-trimethyl dodeca-2, 4 dienoate) and RH 5992 (Tebufenozide, 1,2-dibenzoyl-1-*tert*-butylhydrazide) were obtained as gifts from Dr. D. Cerf (Zoecon Corporation, California, USA) and Dr. T. S. Dhadialla (Rohm and Haas Company, Spring House, Pennsylvania, USA), respectively.

JHA and RH 5992 were dissolved in cold acetone to prepare a solution of 5 μg/μl. The thorax-ligated larvae were treated topically with a single dose of 5 μg JHA or 5 μg RH 5992 on the abdominal tergites using a Hamilton microsyringe. The thorax-ligated larvae treated with a single dose of 1 μl acetone were kept as controls. Testes were dissected from the treated and control larvae after 24 h i.e., on day 2.

RESULTS AND DISCUSSION

Flow cytometric analysis of spermatogenesis in sixth instar larva

The overall pattern of temporal changes in the frequency of cells with different DNA content *viz*, 1C, 2C and 4C is well evident during the developmental period extending from the sixth instar day 0 to day 4 larvae (Fig. 1). The DNA histograms (Figs 2–6) show the frequency of cells (Y-axis) with different DNA contents (X-axis).

The percentage distribution of testis cells of the sixth instar day 0 larvae shows three peaks corresponding to cells with 1C, 2C and 4C DNA content (Fig. 2). Cells intermediate between the 2C and 4C peaks represent the cells in S-phase. In the sixth instar larvae the frequency of 2C cells increased on day 0 and day 1 larvae (Figs 2 and 3), which later on, declined in the day 2 larvae (Fig. 4). These findings corroborate our earlier histological observations (Venugopalan *et al.*, 1994), showing spermatogonial multiplication early in the sixth instar larval development. Since there is a slight increase in the frequency of 1C cells and a significant increase in the population of 2C cells during transition from day 0 to day 1, it can be asserted that the 4C cells in the sixth instar day 0 larva are of two categories. Majority of them are in the G2/M phase of mitotic division of spermatogonial cells and a few of them represent the primary spermatocytes. Similarly, most of the S-phase cells of the day 0 are in the mitotic S-phase whereas a few cells are in the premeiotic S-phase.

In the day 2 larvae, the proportion of 2C cells declined steeply and the population of 1C cells increased considerably (Fig. 4). Most of the S-phase cells in the day 1 larvae might therefore be in the premeiotic S-phase. The 4C population of cells represents the primary spermatocytes. The proportion of 2C cells declined on day 3 with corresponding increase in the frequency of 1C cells (Fig. 5). Most of the 4C cells on day 2 are, therefore, the primary spermatocytes undergoing meiotic division. On day 3 and day 4, the proportion of the cells in S-phase and the frequency of 4C cells was less. The proportion of 1C cells was maximum on day 4 (Fig. 6). On day 4, the
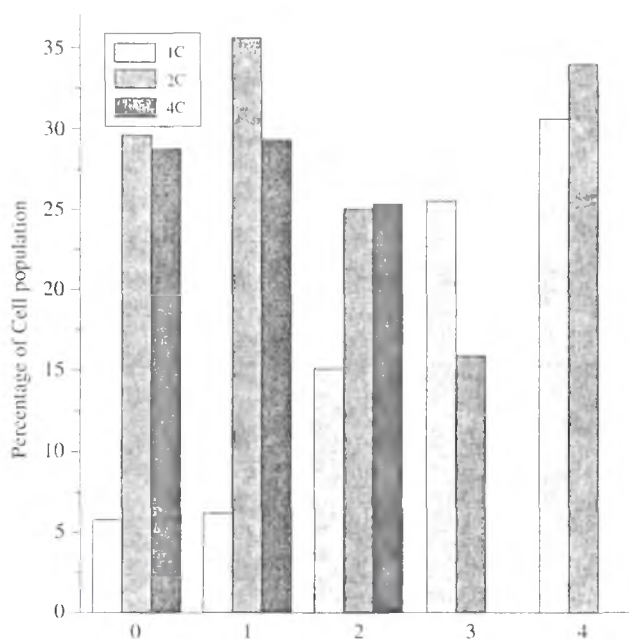
T. M. Benny and V. S. K. Nair

FIGURE 1. Percentage distribution of 1C, 2C and 4C cells in the tests during different days of sixth instar development.

frequency of 2C cells again showed a slight increase, which might be due to the second phase of cell proliferation occurring during the onset of apyrene spermatogenesis (Venugopalan *et al.*, 1994).

The cells with 1C DNA (spermatids) were present even from day 0 stage of the sixth instar larval development suggesting commencement of spermiogenesis during later phase of the fifth instar larval development of *S. mauritia*. It is generally accepted that in lepidopterans juvenile hormone (JH) is present at relatively high levels in the penultimate larval instar and in the early half of last larval instar. JH level then declines to undetectable level by the end of feeding period (Jones *et al.*, 1981; Edwards *et al.*, 1995). Our experimental studies (Santha and Nair, 1986, 1987; Balamani and Nair, 1989, 1991, 1992) corroborate these findings of earlier workers. The present studies suggest that mitotic division of gonial cells occur in the presence of JH. Further meiotic divisions, which take place in the last instar larva, occur during the declining titer of JH.

Effects of JHA and RH 5992 on spermatogenesis

The proportion of 1C, 2C and 4C cells in the testis of thorax-ligated larvae is much lower than that of unligated larvae (Fig. 7). Similarly, the frequency of cells in S-phase also remained low in ligated larvae, which might be due to the absence of various hormonal factors that drive the cells through a cell cycle. The slight increase in the
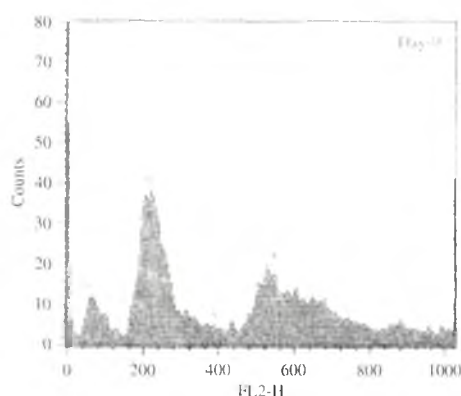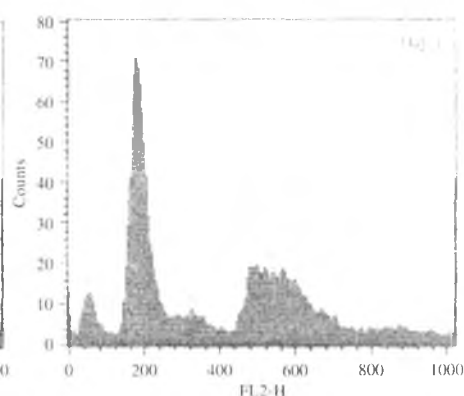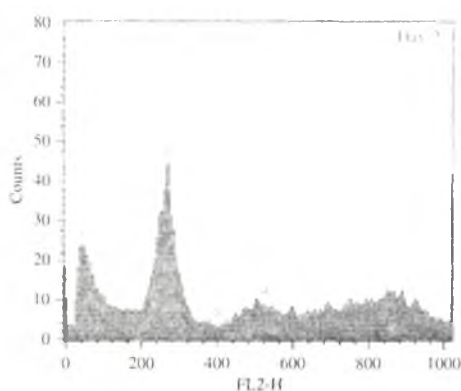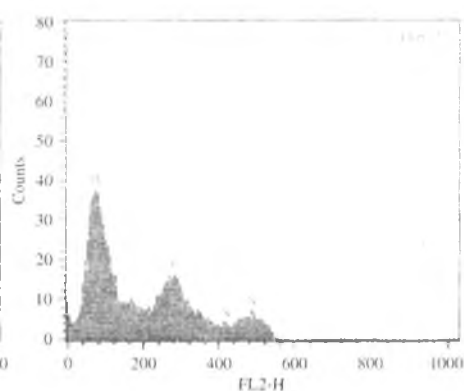
Fig. 2

Fig. 3

Fig. 4

Fig. 5

FIGURES 2-5. Flow cytometric analysis of spermatogenesis in *Spodoptera mauritia*. The histograms show the frequency of cells (Y-axis) with different DNA contents (X-axis).

population of 1C cells indicates that the cells, which are already committed to meiosis, underwent the division process.

In thorax-ligated JHA treated larvae, the proportion of 2C cells was greater than that of control larvae (Fig. 8) suggesting that the JHA has no effect on the basal level of mitotic activity in gonial cells. The proportion of the cells in S-phase and the frequency of 1C cells were, however, less in the JHA treated larvae in comparison to that of controls. The proportion of 4C cells was, however, greater than that of the control and unligated larvae (Fig. 10). The reduction in the proportion of 1C cells and increase in the frequency of 4C cells indicate an inhibitory effect of JHA on meiotic division. On
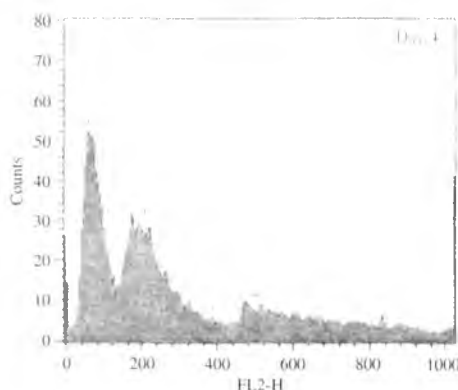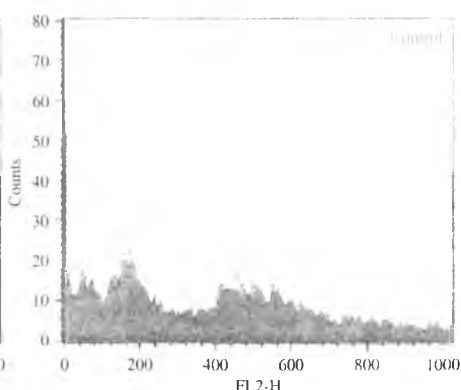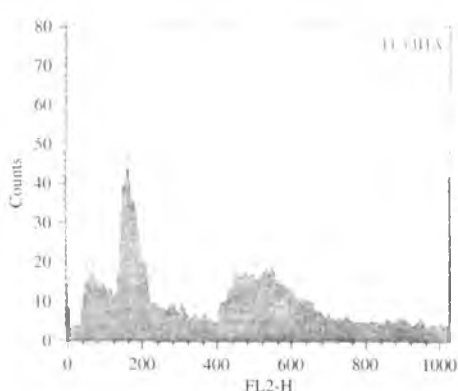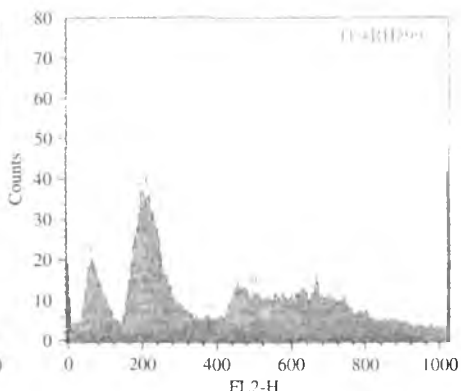
FIGURES 6-9. Flow cytometric analysis of spermatogenesis in *Spodoptera mauritia*. The histograms show the frequency of cells (Y-axis) with different DNA contents (X-axis).

the other hand, JHA showed no effect on the basal level of mitotic activity of germ cells.

In the testis of thorax-ligated RH 5992 treated larvae the proportion of 2C and 4C cells is very high in comparison to that in the controls (Fig. 9). The proportion of 2C and 4C cells is found higher than those of unligated day 2 larvae (Fig. 10) which demonstrate that the ecdysone agonist RH 5992 speed up the cell cycling and the flow of cells from G1 to S and G2 phases. Obviously, the RH 5992 accelerates the mitotic division of spermatogonial cells. The proportion of 1C cells in the testes of treated larvae is almost similar to that of the control larvae, suggesting that RH 5992 has no effect on meiotic division.
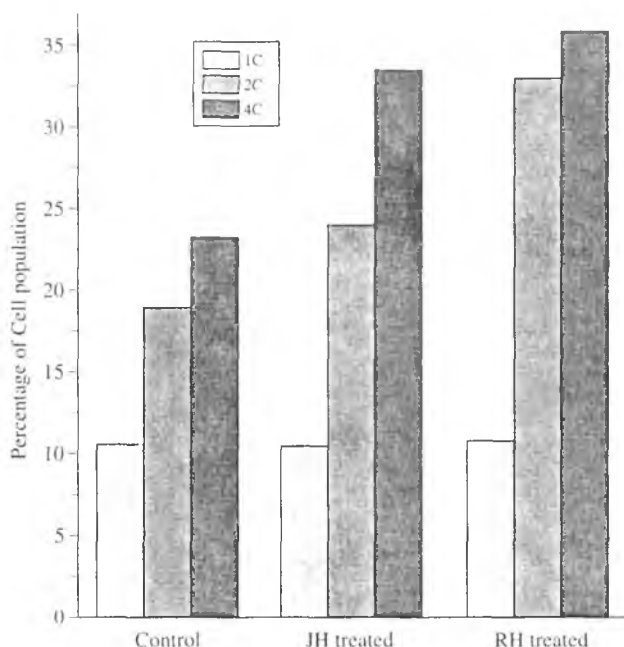
FIGURE 10. Effects of treatments of JHA and RH 5992 on the percentage distribution of 1C, 2C and 4C cells in the tests of ligated larvae.

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Development of resistance to Cry1Ac MVP II in diamondback moth *Plutella xylostella* (L.) and its inheritance

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ABSTRACT: Resistance of *Plutella xylostella* to Cry1Ac MVP II formulation was
studied in laboratory. The median lethal concentrations of Cry1Ac MVP II for the
selected and unselected population (from F_4 to F_9) were 0.70 and 1.70 ng/cm^2,
respectively. The increase in resistance over five generations was 3.5 fold. Mass
crosses were done between the selected and unselected population and LC_{50} of
F_1 hybrid was 1.02 ng/cm^2 which was higher than that of unselected population
(0.50). The degree of dominance was 0.13, whereas the estimate of dominance was
0.56, which indicated incomplete dominance of Cry1Ac resistance in *P. xylostella*.
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KEYWORDS: *Plutella xylostella*, Cry1Ac, resistance, dominance

INTRODUCTION

Transgenic crops are being evolved with emphasis on pest/disease resistance with a
view to overcoming the adverse effects of intensive use of chemicals. Such crops have
been evolved against the serious pest of cruciferous plants using Bt genes. The Cry
proteins produced by the introduced genes are toxic to the target insect pests. The
emergence of strains of pests resistance to the toxicants have been reported recently
indicating a possible limitation of the technology in plant protection.

The ability of resistant *Plutella xylostella* to survive on transgenic canola and
broccoli expressing *cry1Aa* and *cry1Ac* was reported (Suresh Ramachandran *et al.*,
1998; Tang *et al.*, 1999). In this paper the results of a laboratory study on the highly
toxic Bt insecticidal crystal protein Cry1Ac MVP II resistance in *P. xylostella* are
reported.

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MATERIALS AND METHODS

Toxin

The Cry1Ac MVP II formulation from Mycogen Inc. USA was used in the present investigation.

Insects

A single laboratory colony was established from *P. xylostella* adults collected from cabbage and cauliflower field near Akola and Akot, Maharashtra. The field population was not previously exposed to Bt formulations. They were reared in the laboratory on mustard seedlings up to F_4 generation for establishing homogenous population (Shelton *et al.*, 1993). The population was maintained and all experiments were carried out at temperature $28 \pm 1°C$, 70% RH and photoperiod of 14 h light and 10 h dark.

Insect bioassay

Bioassays were carried out using 5 cm diameter cabbage leaf disc (Tabashnik *et al.*, 1991). The leaves were first washed with distilled water containing 0.1% Triton X-100 thoroughly and dried. The required quantity of toxin was diluted in sterile distilled water and spread uniformly over leaf discs (both sides) with the help of glass rod. The leaf discs were placed in individual plastic petridishes containing moistened filter paper (Sayyed *et al.*, 2000). Ten third instar larvae were placed on each disc and each treatment was replicated 4–6 times. The mortality was recorded after every 24 hrs up to 96 hrs. The mortality data were subjected to probit analysis for the estimation of median lethal concentration (Finney, 1971).

Selection of resistant population

The selection was initiated from F_4 generation using single concentration method (Tabashnik *et al.*, 1993). About two hundred third instar larvae were selected at 0.90 ng/cm^2 Cry1Ac. The fresh cabbage leaves were added to the petriplates after 2 days. The survivors were separated on 5th day and reared. They were forwarded to the next generation with the increased dose of toxin (1.08 ng/cm^2). A laboratory susceptible population was maintained without exposure. The LC_{50} for the F_9 generation was worked out.

Dominance of resistance

To estimate the degree of dominance 20 adults from selected and unselected population which consisted of both male and female were crossed (McGaughey and Beeman, 1988) and the median lethal concentration was ascertained for F_1 hybrid. Response to selection (R) was calculated using the median lethal concentration of F_4 (unselected) and F_9 (selected) population. The reciprocal of the 'R' gives the number of generations required for 10 fold increase in the LC_{50} values. The degree of dominance (D) was calculated using $D = (2X_2 - X_1 - X_3)/(X_1 - X_3)$ where, X_1, X_2 and X_3 are the

log LC_{50}'s for the resistant homozygotes, heterozygotes and susceptible homozygotes, respectively. The estimate of dominance (h) calculated using the formula $(D + 1)/2$, where D is the degree of dominance (Sayyed *et al.*, 2000).

RESULTS AND DISCUSSION

The median lethal concentration for the selected and unselected population at F_9 was 1.76 and 0.56 ng/cm^2 (Table 1). The increase in resistance over generations was 3.5 fold. This increase in the resistance was low compared to the earlier reports (Sayyed *et al.*, 2000; Tabashnik, 1994). In most of the earlier studies the LC_{50} of resistant population was compared with the LC_{50} of the most susceptible "ROTH" population which was maintained in the laboratory for more than 150 generations without exposure to toxicants. In the present study the population used for calculating resistance was only 9 generation susceptible. The LC_{50} of toxicant to F_1 hybrid was 1.02 ng/cm^2 (Table 1), which was higher than that of unselected population. The degree of dominance was 0.13, where as the estimate of dominance was 0.56 (Table 1). This indicates the incomplete dominance of Cry1Ac to *P. xylostella*. These findings were fully in agreement with the previous results (Sayyed *et al.*, 2000; Liu and Tabashnik, 1997). The recessive character of Cry1Ac resistance in *P xylostella* was also documented earlier (Tabashnik *et al.*, 1997b). If complete or partial dominance of resistance exist in the population at least two different gene interaction will be present. The estimate of dominance was based on the assumption and hypothesis that the resistant individual are completely homozygous in F_1 progeny. The heterozygotes in a selected population would low the survival rate of F_1 progeny and therefore underestimate the degree of dominance, while the heterozygotes in susceptible population have the opposit effect (Liu and Tabashnik, 1997).

The reciprocal of response of selection (R-Table 1) shows that Cry1Ac requires approximately 12 generations for 10 fold increase in resistance. Sayyed *et al.* (2000) concluded that more number of generations required for 10 fold increase in resistance to Cry toxin, which is in agreement with the present study.

For effective resistance management programme, monitoring, surveillance and early detection of resistant phenotypes in the field populations are important pre requisites in order to initiate timely remedial measures and to evaluate the effectiveness of resistance management strategies. The Bt transgenics forms a important component in integrated pest management. The success of this technology will depend on the sustained susceptibility of the target pests to the Bt strains used in transgenic crops.

Survival and weight gain of *P. xylostella* which had developed more than 1500 fold resistance to foliar sprays of Btk, did not show significant difference between transgenic broccoli and control plants (Tang *et al.*, 1999). Suresh Ramachandran *et al.* (1998) reported that the resistant *P. xylostella* showed no difference between transgenic and non transgenic canola in larval survival, head capsule width at day five, percentage pupation, pupal weight, percentage adult emergence and extent of defoliation (Shelton *et al.*, 1993). Under field conditions, the resistant individuals persists despite high expression of Cry1A toxins and may subsequently contribute

TABLE 1. Response of resistant and susceptible *P. xylostella* and their hybrid progeny to Cry1Ac MVP II.

Population	Generation	LC_{50} ng/cm²	Fiducial limit at LC_{95}	Slope (SE)	RR	D	h	R	n
UNSEL	F_4	0.70	1.71–2.92	3.29 (0.36)	1.39	–	–	–	420
Cry1Ac-SEL	F_9	1.76	2.97–5.30	4.67 (0.66)	3.5	–	–	0.79	240
UNSEL	F_9	0.50	0.43–1.04	3.65 (0.33)	–	–	–	–	240
F_1(Cry1Ac R × S)	F_1	1.02	1.73–3.40	4.41 (0.69)	–	0.13	0.57	–	240

RR - resistant ratio, D - degree if dominance, h - estimate of dominance, R - response to the selection, n - number of insects.

to genetic pool. Eventhough the Bt resistance was not wide spread, modeling studies suggest that the increased selection pressure from commercial release of transgenic Bt plant could cause rapid evolution of resistance unless a proactive approach to resistance management is prescribed.

ACKNOWLEDGEMENT

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Effect of two host plants of *Helicoverpa armigera* (Hübner) on the feeding potential of three chrysopid predators –*Chrysoperla carnea* (Stephens), *Mallada boninensis* (Okamoto) and *Mallada astur* (Banks)

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ABSTRACT: The equal effectiveness of the chrysopid species - *Chrysoperla carnea* (Stephens), *Mallada boninensis* (Okamoto) and *Mallada astur* (Banks) in managing *Helicoverpa armigera* (Hübner) in the sunflower ecosystem has been demonstrated. Release of one chrysopid larva per plant is recommended for *H. armigera* management in the sunflower ecosystem. Chrysopid larvae are ineffective in the pigeonpea ecosystem, where feeding potential was adversely affected, particularly when eggs were present on flowers and pods. However, if chrysopids have to be further evaluated on different varieties of pigeonpea, *C. carnea* or *M. astur* could be tested at the rate of one larva per plant. © 2006 Association for Advancement of Entomology

INTRODUCTION

In India, more than 60 arthropod species have been recorded as predators of *Helicoverpa armigera* (Hübner). In most cases, tri-trophic relationships have not been studied and the role of predators in regulating *H. armigera* populations, either individually or as a group has not been quantified (Manjunath *et al.*, 1989; Romeis and Shanower, 1996). The important predators found feeding on *H. armigera* in India are chrysopids, anthocorids, ants, coccinellids and spiders (Manjunath *et al.*, 1989; Duffield, 1993, 1995). Exclusion experiments have also demonstrated the importance

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of naturally occurring predators in keeping the pest population at low levels (Van Den Berg and Cock, 1993).

In India, chrysopids form an important group of predators. A number of studies have been conducted on the biology, population ecology, feeding potential and rearing of the potential ones such as *Chrysoperla carnea* (Stephens), *Mallada boninensis* (Okamoto), *Mallada astur* (Banks) and *Apertochrysa* sp. (Krishnamoorthy and Nagarkatti, 1981; Patel *et al.*, 1988; Singh *et al.*, 1994; Bakthavatsalam *et al.*, 1994). The type of host plant and its structures can have an impact on predator efficacy (Romeis *et al.*, 1995). It is important for the predators to be able to locate suitable food and oviposition sites if they are to survive and reproduce (Whitman and Nordlund, 1994).

The present investigation was conducted to study the effect of two different host plants, sunflower and pigeonpea on the feeding potential of three important chrysopid species. It was also designed to test different release rates of chrysopid larvae on sunflower and pigeonpea to establish an optimum release rate for *H. armigera* management.

MATERIALS AND METHODS

Feeding potential of chrysopids on *H. armigera* eggs on sunflower and pigeonpea

Pot studies were conducted to study the feeding potential of chrysopids when released on sunflower (*Helianthus annus* L.) and pigeonpea (*Cajanus cajan* (L.) Millsp.) plants infested with *H. armigera* eggs. Potted sunflower (variety – Morden at early flowering stage) and pigeonpea (variety – ES-90 with flowers and young pods) plants were artificially infested with *H. armigera* eggs at 6 and 15 eggs per plant, respectively. Eggs were distributed over the leaves and bracts of sunflower plants and over the leaves, buds/flowers and pods of pigeonpea plants. On the sunflower plants, the topmost three tender leaves and three bracts on three sides of the topmost bloom on each plant were chosen for inoculationg *H. armigera* eggs (1 egg per plant part). On the pigeonpea plants, the topmost five tender leaves, five flowers and five young pods were chosen on each plant and eggs inoculated (1 egg per plant part). Preliminary investigations revealed that second instar chrysopid larvae were most suitable for releases. Hence, second instar chrysopid larvae were released at 1, 2 and 3 individuals per plant. Other naturally occurring general predators were prevented from feeding on *H. armigera* eggs by providing barriers. After 24 h, the eggs on each plant were examined for chrysopid-induced feeding damage. The percentage of eggs fed upon by the chrysopids was calculated. The experiment was repeated for each chrysopid species tested: *C. carnea*, *M. boninensis* and *M. astur*. For each treatment, five sets of 10 plants were used as replications. The percentage values were subjected to angular transformation in order to normalise the data and then subjected to two-way ANOVA.

Effect of different plant structures on feeding potential of chrysopids

The methodology followed in this experiment was identical to the previous experiment, except that the percentage of eggs fed upon by the chrysopid on each plant part (leaf and bract of sunflower plant and leaf, bud/flower and pod of pigeonpea plant) was calculated separately. For this experiment, chrysopid larvae were released at 1 larva per plant (based on the results of the previous experiment). Data on per cent eggs fed on different plant structures of the two host plants was subjected to angular transformation and two-way ANOVA.

Experiments were conducted during September to December. Environmental conditions were recorded at $26 \pm 2°C$ and RH, $60 \pm 10\%$ and 11L: 13D hours. Chrysopid releases were made during evening hours between 4 and 5 PM. *H. armigera* eggs and chrysopid larvae used for these experiments were obtained from the Mass Production Laboratory of the Project Directorate of Biological Control, Bangalore, India.

RESULTS AND DISCUSSION

Feeding potential of chrysopids on *H. armigera* eggs

On sunflower

At different release rates on sunflower plants, *C. carnea* fed on 51.67 to 66.67%, while *M. boninensis* on 61.67 to 63.83% and *M. astur* on 65 to 75% of the eggs. No significant differences were observed among the different chrysopid species and the different release rates or the interaction treatments with reference to their feeding potential on *H. armigera* eggs on sunflower plants (Table 1). The above results indicated that on sunflower, the tested chrysopid species performed equally well. Singh *et al.* (1994), based on laboratory studies, observed that the feeding potential was maximum in *M. boninensis* followed by *Apertochrysa* sp., *M. astur* and minimum in *C. carnea*. The difference observed in the present investigation could be attributed to the influence of the host plant (sunflower) on the feeding potential of the chrysopid species.

The present investigation revealed that the release of 2 or 3 chrysopid larvae (of all the three chrysopid species tested) was not more advantageous in comparison to the release of 1 larva per sunflower plant. Hence it could be suggested that on sunflower plants any one of the three chrysopids - *C. carnea*, *M. boninensis* or *M. astur* could be released at the rate of one second-instar larva per plant. Earlier studies revealed that three releases of first instar larvae of *Brinckochrysa scelestes* (Banks) @1 larva per head at 10 day intervals could provide complete suppression of *H. armigera* on sunflower plants (Venkatesan *et al.*, 1994).

Though all the three chrysopids performed equally well on sunflower plants, it would be ideal to choose *C. carnea* as this predator is found in natural conditions in the sunflower ecosystem and so is well adapted to this ecosystem. The orientational and ovipositional preference of *C. carnea* adults to sunflower plants (Ballal and Singh, 1999) supports the above fact. According to Bakthavatsalam *et al.* (1994), *C. carnea*

TABLE 1. Per cent eggs fed by Chrysopid spp. at different release rates on sunflower

Chrysopid species	Release rates			Mean
	1 per plant	2 per plant	3 per plant	
Chrysoperla carnea	51.67 (46.30)	66.67 (57.35)	62.50 (53.18)	60.28 (52.28)
Mallada boninensis	62.50 (52.61)	61.67 (54.43)	63.83 (54.01)	62.67 (53.68)
Mallada astur	75.00 (62.42)	65.00 (55.27)	74.67 (62.19)	71.56 (59.96)
Mean	63.06 (53.78)	64.45 (55.68)	67.00 (56.46)	

Figures in parentheses are angular transformed values. The differences between treatments were not significant.

TABLE 2. Per cent eggs fed by Chrysopid spp. at different release rates on pigeonpea

Chrysopid species	Release rates			Mean
	1 per plant	2 per plant	3 per plant	
Chrysoperla carnea	38.20 (38.45)	38.00 (37.94)	19.01 (25.65)	31.74 (34.01)[a]
Mallada boninensis	11.60 (17.42)	18.61 (25.84)	10.60 (18.14)	13.61 (20.47)[b]
Mallada astur	25.41 (30.16)	29.02 (31.92)	13.43 (20.05)	22.62 (27.41)[a]
Mean	25.08 (28.68)[a]	28.54 (31.90)[a]	14.35 (21.28)[b]	

Figures in parentheses are angular transformed values.
CD at $P < 0.05$: Species 6.62; Release rate 6.90.

has the highest net reproductive rate as compared to other chrysopids, which is also a point in favour of utilising *C. carnea* as the bio-agent. The fact that this predator is amenable to mass multiplication is an added advantage.

On pigeonpea

The percentages of *H. armigera* eggs consumed or damaged on pigeonpea plants were 19.01 to 38.2%, 10.6 to 18.61% and 13.43 to 29.02% for *C. carnea*, *M. boninensis* and *M. astur*, respectively, at different release rates. Irrespective of the chrysopid species, percentage eggs fed was significantly higher at the release rates of 1 and 2 larvae per plant compared to 3 larvae per plant. Irrespective of the release rates, comparison of the chrysopids' performance on pigeonpea plants, showed that feeding was maximum by *C. carnea* (31.74%) and *M. astur* (22.62%) and significantly lesser by *M. boninensis* (13.61%) (Table 2).

On pigeonpea plants, the per cent eggs fed was low (10.61 to 38%) which indicated the ineffectiveness of the release of chrysopid larvae on pigeonpea plants. However, if further trials have to be conducted on the release of chrysopid larvae on different varieties of pigeonpea in field conditions, *C. carnea* or *M. astur* larvae at the rate of one per plant could be suggested.

TABLE 3. Per cent eggs fed by Chrysopid spp. on different plant parts of sunflower

Chrysopid species	Sunflower plant part		Mean
	Leaf	Bract	
Chrysoperla carnea	53.33 (46.47)	57.78 (50.60)	55.56 (48.54)
Mallada boninensis	64.44 (54.15)	68.89 (56.73)	66.67 (55.44)
Mallada astur	71.11 (62.15)	82.22 (73.13)	76.67 (67.64)
Mean	62.96 (54.26)	69.63 (60.15)	

Figures in parentheses are angular transformed values. The differences between treatments were not significant.

TABLE 4. Per cent eggs fed by Chrysopid spp. on different plant parts of pigeonpea

Chrysopid species	Pigeonpea plant part			Mean
	Leaf	Flower	Pod	
Chrysoperla carnea	59.00 (52.26)	6.00 (12.75)	26.00 (26.45)	30.33 (30.49)[a]
Mallada boninensis	12.00 (17.49)	9.40 (15.78)	6.40 (13.11)	11.10 (15.46)[b]
Mallada astur	47.00 (43.54)	10.00 (16.17)	32.00 (29.95)	29.67 (29.89)[a]
Mean	39.33 (37.76)[a]	8.47 (14.90)[b]	21.47 (23.17)[b]	

Figures in parentheses are angular transformed values.
CD at $P \leq 0.05$: Species 13.08; Plant part 12.09.

Effect of plant structures on feeding potential of chrysopids

Sunflower

No significant differences were observed in the feeding potential of the three different species of chrysopid larvae or when *H. armigera* eggs were present on the leaves or bracts of sunflower plants (Table 3). The tested chrysopid species could feed on 53.33 to 82.22% *H. armigera* eggs on sunflower plant parts.

Pigeonpea

Irrespective of the chrysopid species tested, maximum feeding was on the eggs present on leaves (39.33%), which was significantly more than that on flowers (8.47%) and pods (21.47%) and the latter two were statistically on par. Irrespective of the plant part, when the three chrysopid species were compared, maximum feeding was observed in the case of *C. carnea* and *M. astur* (30.33 and 29.67%, respectively), which was significantly superior to *M. boninensis* (11.1%) (Table 4).

The effect of plant parts on predatory behaviour was reported for other predators too. The ant, *Paratrechina longicornis* (Latreille) could remove a large number of eggs from the leaves of pigeonpea plants, but the eggs on flowers, flower buds, flower petals or pods suffered significantly less predation (Romeis *et al.*, 1995). This was explained to be due to the type and distribution of trichomes on different pigeonpea plant parts/structures and the same explanation could hold true in the present experiment.

However, the sunflower plant parts had no influence on the feeding potential of chrysopids. A similar trend was observed in the behaviour of adult *C. carnea*, which distributed its eggs uniformly on sunflower plant parts, while on pigeonpea, though few eggs were laid on leaves and pods, there was no egg laying on flowers (Ballal and Singh, 1999).

The higher rate of feeding of *H. armigera* eggs by chrysopids on sunflower than pigeonpea indicated the effect of host plants on the efficiency of chrysopid larvae as biocontrol agents. During the course of the present studies, the observation that the chrysopids were found to remain on the sunflower heads even a week after release, while they were never found to remain on the pigeonpea plants indicated that the predator could survive better on the former. It can be inferred from this experiment that chrysopids, especially *C. carnea*, are efficient predators in the sunflower ecosystem for the management of *H. armigera*. However, further studies are needed to investigate the response of chrysopids to different varieties of pigeonpea, and to explore the possibility of using kairomones for improving the performance of these predators in pigeonpea ecosystem.

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Efficacy of the egg parasitoids, *Trichogramma* spp. for the management of *Eublemma amabilis* Moore (Lepidoptera: Noctuidae) — a predator of Indian lac insect

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ABSTRACT: Three species of the egg parasitoids namely, *Trichogramma achaeae* Nagaraja and Nagarkatti, *T. exiguum* Pinto and Planter and *T. ostrinae* Pang *et* Chen were evaluated in field for management of *Eublemma amabilis* Moore (Lepidoptera: Noctuidae), the major insect predator of Indian lac insect *Kerria lacca* Kerr. Experiments were carried out on two lac host plants namely, *Butea monosperma* (Lam) Taub and *Albizzia lucida* Benth for *rangeeni* and *kusmi* biotypes of lac insects respectively. Significant suppression in the incidence of *E. amabilis* was observed over control with release of 75 egg parasitoids per plant in case of *kusmi* and *rangeeni* biotypes. The increase in lac crop yield was significantly high with respect to control in case of all the three egg parasitoids with 100 parasitoids per plant for *kusmi* biotype. However, for *rangeeni* only *T. exiguum* and *T. ostrinae* could provide significant difference in yield. © 2006 Association for Advancement of Entomology

KEYWORDS: Egg parasitoids, *Trichogramma*, *Eublemma amabilis*, lac crop, *Kerria lacca*

The Indian lac insect *Kerria lacca* (Kerr) is susceptible to a number of insect pests causing significant loss in quality and quantity of yield. The contribution of lac to the tribal economy in rural belts is significantly high in the states of Jharkhand, West Bengal, Chhattisgarh, Madhya Pradesh and Maharashtra. A large number of insect predators and parasitoids damage the lac crop and the annual loss to cultivation is estimated to be 30–40% (Glover, 1937; Malhotra and Katiyar, 1979). *Eublemma amabilis* Moore (Lepidoptera: Noctuidae) is one of the key predatoer of lac insect, affecting the lac crop under field condition. The current study is an effort to explore the scope of biological control of *E. amabilis* in lac ecosystem utilizing some promising egg parasitoids. The eggs of *E. amabilis* alone are exposed and are accessible to parasitoids, as the larval and pupal stages remain confined in the tunnels formed within the lac encrustation. Several species of *Trichogramma*

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(Hymenoptera: Trichogrammatidae) have been reported to parasitize the eggs of lepidopteran insect predators in lac ecosystem (Sushil *et al.*, 1995, 1999, 2000). Three species, *Trichogramma achaeae* Nagaraja and Nagarkatti, *T. exiguum* Pinto and Planter and *T. ostrinae* Pang *et* Chen were evaluated under field condition on *rangeeni* and *kusmi* crop for assessing their efficacy in the management of *E. amabilis*.

The parasites were reared in laboratory on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) as per standard procedures. The eggs were collected on paper cards and sterilized in UV light for 45 min, and exposed to the parasitoids for egg laying. The cards containing parasitised eggs were kept under laboratory condition at room temperature for 5–6 days and then they were stored in refrigerator for later use.

The rainy season *rangeeni* crop and winter season *kusmi* crop were raised on two common host plants namely *Butea monosperma* (Lam) Taub, and *Albizzia lucida* Benth in field. The three species of egg parasitoids were released by stapling the trichocards on the under surfaces of leaves near about the lac encrusted twigs/shoots of the lac host trees on 30 days and 60–65 days after crop inoculation which normally corresponded with the oviposition period of the lepidopteran predator *E. amabilis*. Three doses, *viz.* 50, 75 and 100 parasitised eggs per tree, corresponding to the release rate of 50,000 to 1,00,000 parasitoids per hectare were released with each dose being replicated five times. For *B. monosperma* the release rate of 50 eggs per plant was avoided owing to the large size of the tree. Five plants far away from the site of release were kept as control. The area of experiment was isolated and no insecticides were applied in the adjacent plots. Samples in the form of lac encrustation measuring 1.5 meters were collected by following stratified destructive random sampling method at the time of crop maturity. These samples were caged in 60-mesh nylon net for a month providing sufficient period of time for the parasitoids and predators to emerge out from the lac encrustation. Cages were opened and predators and parasites inside each bag were identified and counted. The yield per 100 g broodlac was ascertained from the treated and control plots.

The results of egg parasitoid released *kusmi* and *rangeeni* lac culture is summarized in Table 1. Significant suppression in the population of *E. amabilis* was observed with the release of all the three species of *Trichogramma* at the dose of 75 and 100 egg parasitoids per tree in comparison to control for both *kusmi* and *rangeeni* strain cultivated on *B. monosperma* and *A. lucida* respectively. In case of *T. ostrinae* a dose of 50 egg parasitoid per tree was able to cause effective suppression. However, there was no significant difference in incidence of *E. amabilis* between the doses 75 and 100 egg parasitoids per tree in all the cases. Hence a dose of 75 egg parasitoids per tree is sufficient to suppress the population of *E. amabilis* in case of both the winter season *kusmi* and rainy season *rangeeni* lac crop. The efficacy in checking the population of *E. amabilis* with the respective release doses was in conformity with the dose of other egg parasitoids evaluated in lac ecosystem earlier (Bhattacharya *et al.*, 2003). The re-emergence of released parasitoids was low and could not be correlated with the population of *E. amabilis*.

TABLE 1. Effect of *Trichogramma achaeae, T. exiguum and T. ostrinae* on population of *Eublemma amabilis* on rainy season *rangeeni* crop raised on *Butea monosperma* and *kusmi* crop raised on *Albizia lucida*

Parasitoid	Dose	Number of E. amabilis/1.5 m stick		Parasitoid emerging per 1.5 m stick		Yield (kg) per 100g input	
		Rangeeni	*Kusmi*	*Rangeeni*	*Kusmi*	*Rangeeni*	*Kusmi*
T. achaeae	50	–	8	–	0	–	0.200
	75	4	5	0	0	0.183	0.516
	100	4	3	0	0	0.283	0.766
Control		15	11	0	0	0.116	0.123
CD at 5 %		5.88	5.46			1.74	0.31
T. exiguum	50	–	8	–	0	–	0.316
	75	2	3	0	0	0.7	0.633
	100	1	2	1	0	1.05	1.083
Control		16	11	1	0	0.35	0.350
CD at 5 %		5.70	3.76			0.213	0.09
T. ostrinae	50	–	8	–	0	–	0.333
	75	3	5	0	1	0.233	0.483
	100	3	3	0	0	0.333	1.366
Control		15	12	0	1	0.166	0.150
CD at 5 %		5.88	2.2			0.085	0.26

There was significant increase in yield between control and 75 as well as 100 egg parasitoids per tree in case of *T. exiguum* and *T. ostrinae* for *rangeeni* strain and with all the parasitoids in *kusmi* strain. The difference between 75 and 100 egg parasitoids was also significant with all the three parasitoids for the *kusmi*. However, in case of *rangeeni* only two egg parasitoids *T. exiguum* and *T. ostrinae* could provide significant difference in yield between 75 and 100 egg parasitoids per tree. Hence the release of 100 egg parasitoids per tree can be recommended for rainy season *rangeeni* lac crop on *B. monosperma* and winter season *kusmi* lac crop on *A. lucida*. Though the release dose of 75 egg parasitoids was sufficient to suppress the population of *E. amabilis*, the quantitative increase in yield with the releases of 100 egg parasitoids indicates that the latter dose is more suitable. Based on the study the relative performance of these egg parasitoids can be expressed as *T. ostrinae* > *T. achaeae* > *T. exiguum* for *kusmi* and *T. exiguum* > *T. ostrinae* for rangeeni. The study also reveals disproportionate increase in yield in various treatments unrelated to the suppression in the population of *E. amabilis*. The finding may be attributed to several other biological factors exerting their influence in lac ecosystem. The incidence of another lepidopteran insect predator *P. pulverea* was negligible in all the treatments and was not considered under the present study.

ACKNOWLEDGEMENT

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Icfrealeyrodes indica, a new genus and species of whitefly (Hemiptera: Aleyrodidae) from India

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ABSTRACT: *Icfrealeyrodes indica*, a new genus and species of whitefly (Hemiptera: Aleyrodidae) are described from India, where the species is found on *Syzygium* sp. in Karnataka. © 2006 Association for Advancement of Entomology

KEYWORDS: Taxonomy, Aleyrodidae, *Icfrealeyrodes*, *new genus*

The taxonomy of whiteflies is largely based on nymphal characters and on a world basis there are about 1420 known species of whiteflies in two subfamilies (Ko, 2001). The known Indian fauna of whiteflies comprises 271 species in 56 genera. This paper describes a new genus and species of whitefly found on *Syzgium* sp. in Karnataka.

Description

Icfrealeyrodes Dubey and Sundararaj gen. nov.

Type species

Icfrealeyrodes indica gen. nov. by original designation

Diagnosis   White with secretion of little wax; broadly elliptical, deeply constricted at thoracic and caudal tracheal pore regions with inner teeth; cephalic, first abdominal and caudal setae present; submargin completely separated from margin by a well defined submarginal furrow; longitudinal and transverse moulting suture reaching submarginal furrow; subdorsum granulated with polygonal markings; submedian row of papillae present. Vasiform orifice and operculum triangular, wider than long, lingula concealed.

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Etymology

Names after Indian Council of Forestry Research and Education (ICFRE), the parental organization of the Institute of Wood Science and Technology where the study was carried out.

Comments

This genus is close to *Vasantharajiella* David (2000) in having complete submarginal furrow separating submargin from dorsum, but it differs in possessing first abdominal setae and deeply invaginated thoracic and caudal tracheal pores with inner teeth, in the absence of submarginal setae and lack of stipples in thoracic and caudal tracheal folds. This genus is also closely related to *Asialeyrodes* Corbett (1935) in which the submarginal furrow separating dorsum is incomplete posteriorly and thoracic tracheal pores are without inner teeth.

Icfrealeyrodes indica sp. nov. (Fig. 1)

Puparium: White, with secretion of little wax; broadly elliptical, deeply constricted at thoracic and caudal tracheal pore regions, broadest at first abdominal segment, 0.69–0.73 mm long, 0.77–0.80 mm wide, found singly on under surface of leaves. Margin crenulate, 14–15 crenulations in 0.1 mm; thoracic and caudal tracheal pore regions deeply invaginated with internal teeth; anterior and posterior marginal setae respectively, 10 μm and 6 μm long.

Dorsum: A row of minute submedian papillae extending from cephalothorax to abdomen. Submargin separated from dorsal disc by a well defined complete furrow not interrupting even at caudal region, submargin with polygonal markings. Subdorsum granulated with polygonal markings. Submedian pockets present on all cephalothoracic and abdominal segments. Submedian depression present on all cephalothoracic and abdominal segment sutures. Longitudinal and transverse moulting sutures reaching submarginal furrow. Median length of abdominal segment VII much shorter than VIII. Pores and porettes evident. Pockets not contiguous (Fig. 1).

Chaetotaxy: Cephalic setae 5 μm long, first abdominal setae 7 μm long, caudal setae 9 μm long and eighth abdominal setae not discernible.

Vasiform orifice:

Triangular, 22–23 μm long, 29–30 μm wide; operculum triangular, 16–17 μm long, 20–21 μm wide. Lingula concealed. Thoracic and caudal tracheal furrows absent.

Venter: A pair of ventral abdominal setae 14 μm long, 28 μm apart. Thoracic tracheal folds not indicated and caudal tracheal fold indicated without stipples. Antennae reaching base of prothoracic legs.
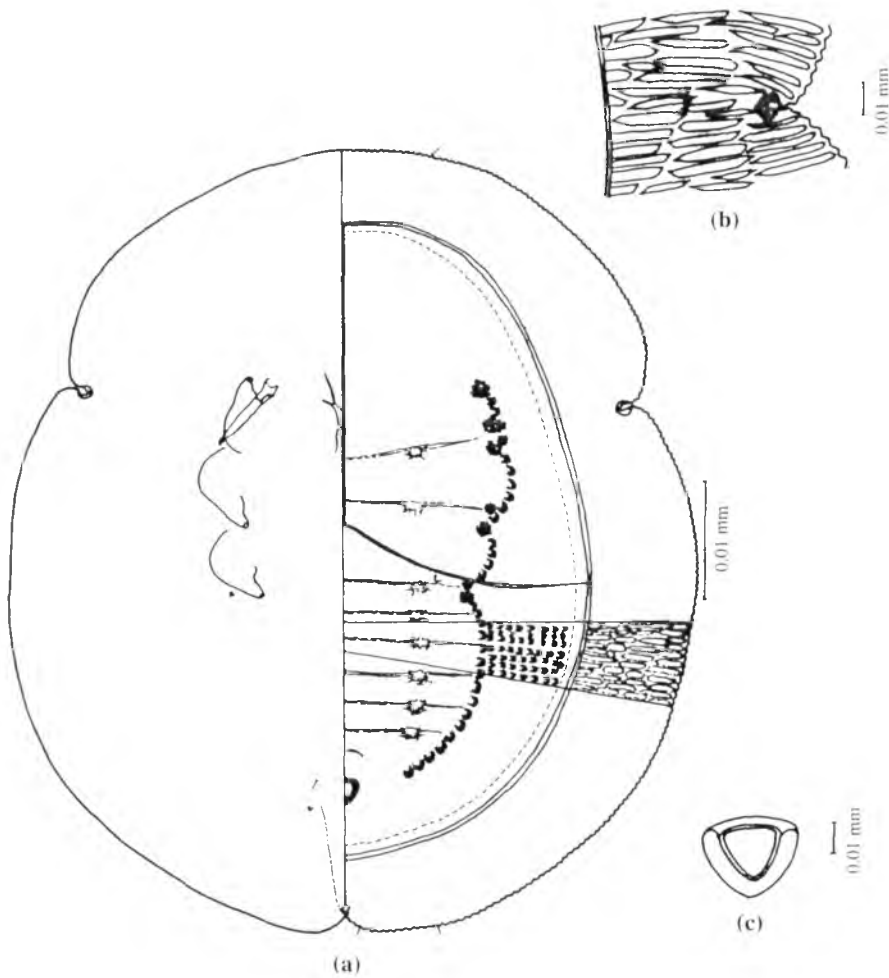
FIGURE 1. *Icfrealeyrodes indica* sp. nov.: a, puparium; b. margin; c. vasiform orifice.

Materials examined

Holotype puparium, India: Karnataka: Yana, on *Syzgium* sp. (Myrtaceae), 17.ii.2001, A. K. Dubey, deposited in Forest Research Institute, Dehra Dun, India. Paratype, one puparium, data same as for holotype, deposited in The Natural History Museum, London, United Kingdom.

Host: Syzygium sp.

Distribution:

India: Karnataka.

Etymology:

Named after India.

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Compatibility of plant disease antagonists *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. Fr. with entomopathogenic fungi of horticultural crop pests

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ABSTRACT: The compatibility of the plant disease antagonists, *Trichoderma harzianum* and *T. viride* with *Verticillium lecanii, Metarhizium anisopliae, Paecilomyces farinosus, Beauveria brongniartii, B. bassiana*, potential entomopathogens of horticultural pests was evaluated under *in vitro* conditions, by dual culture technique. Results indicated that *T harziarum* and *T. viridae* significantly inhibited the mycelia growth and spore yield of the entomopathogens. The present study indicates need for assessing the interaction of beneficial biological control agents used in integrated crop protection technologies. © 2006 Association for Advancement of Entomology

KEYWORDS: *Trichoderma harzianum, Trichoderma viride*, entomofungal pathogens and compatibility

The plant disease antagonists, *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers.: Fr. are widely recommended for control of plant diseases in horticultural crops, by virtue of their high efficacy, broad-spectrum activity, easy mass multiplication methods and metabolic versatility (Papavizas, 1985; Soundarababu, 1998). Similarly, the entomopathogens like *Verticillium lecanii* (Zimm.) Vigeas, *Metarhizium anisopliae* (Metsch.) Sorok, *Paecilomyces farinosus* (Holmskiod) Brown and Smith, *Beauveria brongniartii* (Sacc.) Petch and *Beauveria bassiana* (Balsamo) Vuillemin. are also recommended as potential bio-pesticides for the management of economically important horticultural pests. With increased emphasis on biological controls in the recent years, simultaneous application of plant disease antagonists and entomopathogens is common. This could result in antagonistic interactions perhaps making introduction of a biological controls agent impractical (Krieg, 1971). The present study was carried

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out to determine the interactions between the plant disease antagonists and selected entomofungal pathogens as mentioned above.

The study was carried out under *in vitro* conditions by following dual culture technique (Morton and Straube, 1955). The radial mycelia growth was measured at weekly intervals for two weeks. Per cent inhibition of mycelia growth over control was calculated using the formula given by Vincent (1927).

To determine the conidia yield, the Petri plate was flooded with sterile water, conidia from the surface was scraped using a spatula and grounded gently in mortar and pestle, with 50 ml of sterile water. The solution filtered through sterile muslin was centrifuged at $3000 \times g$ for 15 min. at $4\,^{\circ}\mathrm{C}$, washed with 15 ml cold sterile distilled water and resuspended in 10 ml cold sterile distilled water. By using an improved Neubauer double ruled haemo-cytometer and phase contrast microscope at a magnification of 600x the spore yield was recorded and the results were expressed in number of spores per milliliter to determine the overall effect (Lawrence, 1997). Compatibility was decided finally based on the production of conidial spores.

The experiment was carried out with complete randomized design, with three replications per treatment. The resultant data was subjected to analysis of variance.

The compatibility of *T. harzionum* and *T. viride* on the mycelia growth and conidiation of entomofungal pathogens are discussed below.

Presence of plant antagonists *T. harzianum* significantly inhibited the growth of all entomopathogens screened. A cumulative mycelia growth of 0.96, 1.93, 2.15, 1.85 and 2.03 cm was recorded in dual culture plates of *T. harzianum* and *V. lecanii, M. anisopliae, P. farinosus, B. brongniartii and B. bassiana* on the 14th day after inoculation (Table 1) that accounted for 62.5, 51.6, 59.2, 64.6 and 54.0 per cent inhibition respectively (Table 2).

A similar suppressing property was observed for *T. viridae* towards the screened entomoapthogens. A maximum of 0.95. 1 .70, 2.15, 1.83 and 1.96 cm of radial mycelia growth occurred in dual cultures of *T. viridae* and entomopathogens that accounted for 55.1, 57.2, 59.2, 63.5 and 65.1 percent inhibition of *B. bassiana, M. anisoplicae, P. farinosus, V. lecanii* and *B. brongniartti* respectively (Tables 1 and 2).

Corresponding with the inhibition in mycelia growth a significant reduction in the sporulation of the entomopathogens was also observed in dual culture plates of both plant antagonists. A maximum of 18.7, 2.7, 25.3, 7.7and 40.6×10^6 spore/milliliter was recorded from single culture plates of *V. lecanii, M. anisopliae, P. farinosus, B. brongniartii* and *B. bassiana* (Table 1). The corresponding figures for the dual cultures with *T. harzianum* was 5.3, 0.03, 1.5, 1.7 and 55.3×10^6 spore/milliliter and 5.2, 0.02, 1.4, 1.07 and 47.3×10^6 spores/ml for *T. vridae*. The spore yield recorded in dual culture plates of both plant antagonists were significantly lower than the spore yield recorded from culture plates of the respective entomopathogens.

The present study indicates that both plant disease antagonists have a prolonged inhibitory effect on the growth and sporulation of the screened entomopathogens. Vestergaard *et al.* (1995) reported that *M. anisopliae*, a potential biological control agent for western flower thrips, *Frankiniella occidentalis* survived longer on sterile

TABLE 1. Mean radial mycelia growth of entomofungal pathogens in presence of *Trichoderma harzianum* and *T. viridae*

Treatment	Mean radial growth (in cm)		Conidia ($\times 10^6$) per ml	
	7th day	14th day	7th day	14th day
V. lecanii	1.93^c	2.60^d	0.68^e	18.7^e
V. lecanii + T. harzianum	0.86^f	0.96^h	0.41^{ef}	05.3^g
V. lecanii + T. viride	0.83^f	0.95^h	0.40^{ef}	05.2^g
M. anisopliae	2.00^c	3.98^c	0.06^f	02.7^h
M. anisopliae + T. harzianum	1.45^{de}	1.93^f	0.02^f	0.03^i
M. anisopliae + T. viride	1.33^e	1.70^g	0.02^f	0.02^i
P. farinosus	2.50^a	5.28^a	0.81^e	25.3^d
P. farinosus + T. harzianum	1.95^c	2.15^e	1.27^d	01.5^{hi}
P. farinosus + T. viride	1.96^c	2.15^e	1.22^{de}	01.4^{hi}
B. brongniartii	2.50^a	5.23^a	0.82^e	07.7^f
B. brongniartii + T. harzianum	1.35^e	1.85^f	0.79^e	01.7^h
B. brongniartii + T. viride	1.23^e	1.83^{fg}	0.69^e	1.07^{hi}
B. bassiana	2.18^b	4.40^b	79.3^a	40.69^a
B. bassiana + T. harzianum	1.55^d	2.03^{ef}	31.3^b	55.3^b
B. bassiana + T. viride	1.50^d	1.96^f	25.7^c	47.3^c
CD@0.05	0.12	0.15	0.43	1.6

TABLE 2. Per cent inhibition of plant antagonists *T. viridae* and *T. harziarum* on mycelial growth of entomopathogens

Treatment	Per cent inhibition over control	
	T. viridae	*T. harziarum*
V. lecanii	63.5	62.5
M. anisopliae	57.2	51.6
P. farinosus	59.2	59.2
B. brongniartii	65.1	64.6
B. bassiana	55.1	54.0

potting compost than in non sterile media; presumably under field conditions soil antagonists limit the survival of fungal inocula. Similarly, application of these plant antagonists could mask the growth and sporulation of the entomopathogens, thereby reducing their potentiality as effective biopesticides. There is a need to carry out further detailed studies in instances where they are applied simultaneously for crop protection.

ACKNOWLEDGEMENTS

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Hitherto unknown palpimanid spider (Araneae: Palpimanidae) from India

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ABSTRACT: *Otiothops namratae* sp. nov. is described from four males and seven females collected from Gujarat, India. Spiders of this family have not until now been recorded from India. © 2006 Association for Advancement of Entomology

KEYWORDS: spider, palpimanid, *Otiothops*, India

Palpimanidae is a poorly known spider family. The spiders of this family are characterized by having a red cephalothorax, more or less dark, large, with almost parallel margins and with a great cephalic development. The appendixes have the same color of the cephalothorax and the first pair of legs is very strong. These spiders have slow movements and raise the first pair of legs when moving. The abdomen is oval and has a ventral shield (scutum) covering the epigastric area. They live in leaf litter or under stones in dry soils.

Palpimanids are typical haplogyne spiders; the male palp lacks haematodocha and is with only a bulb and embolus or with a few additional terminal sclerites. Female has no evident epigynum and lacks separate fertilization pores. Fifteen genera have been recognized of Palpimnaidae family. Species assigned to the genus *Otiothops* far outnumber the species belonging to the remaining fourteen genera (Chickering, 1966; Platnick, 1975, 1976, 1977, 1985, 2004; Platnick *et al.*, 1999). The widely separated posterior median eyes, reduced claw tufts and embolus without accessory terminal sclerites are characters found in no other known palpimanids. Spiders of this family have not until been recorded from India.

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Otiothops namratae **sp. nov. (Figs 1a–g)**

General

Cephalothorax, chelicerae, sternum and legs bright reddish brown; abdomen yellow and purplish. Total length 6.51 ($\pm$0.75) mm, carapace 2.30 ($\pm$0.20) mm long, 1.56 ($\pm$0.13) mm wide abdomen 3.86 ($\pm$0.82) mm long, 2.13 ($\pm$0.11) mm wide (mean and standard error of 11 specimens examined).

Cephalothorax

Longer than wide clothed with pubescence. Thoracic region red and elevated anteriorly. Anterior row of eyes straight or slightly recurved. Posterior row of eyes procurved. Eye rows sub equal in length, occupying only two-third to three-fourth of cephalic width. Anterior medians largest and anterior laterals smallest. Anterior medians separated by less than 1-3 times diameter from posterior laterals. Median ocular quadrangle roughly square. Chelicerae short, flattened and rigid anteriorly with three retro marginal teeth. Endite short, convergent and with serrula. Sternum tuberculated with enlarged lateral and posterior tubercles and raised extensions surrounding coxae. Leg I orange and greatly enlarged. Femur I expanded dorsally twice the height of femora II through IV. Patella enormously longer than tibia. Tibia, metatarsus and tarsus bear thick prolateral scapulae. Metatarsus much shorter and rarely as long as tarsus. Tarsus widened at the tip with reduced claw tufts. All legs devoid of spines. First leg: femur 2.44 mm long; patella 1.86 mm long; tibia 1.13 mm long; metatarsus 0.38 mm long; tarsus 0.63 mm long. Leg formula IV, I, II. III.

Abdomen

Brownish purple with anterior ring-like scutum. Tracheal spiracle slightly anterior to spinnerets surrounded by sclerotized rings. Spinnerets two, short and cylindrical. Colulus absent.

Genitalia

Male palp with globose tibia lacking apophysis, long and thin cymbium and the protruding bulb not protected by alveolus. External genitalia absent. Internal genitalia only with spermathecae and without sclerotized connecting ducts or other elaborations.

Type specimens

Collected from Adhevada, Bhavnagar, Gujarat, India (Coll. K. Gopalakrishna Pillai, 25.05.1985). Holotype 1♀, paratypes 4 ♀s, allotypes 2 ♂s in alcohol deposited at Zoological Survey of India, Southern Region, Chennai (Registration No. In/sp/1&2 dated 24.05.2006).
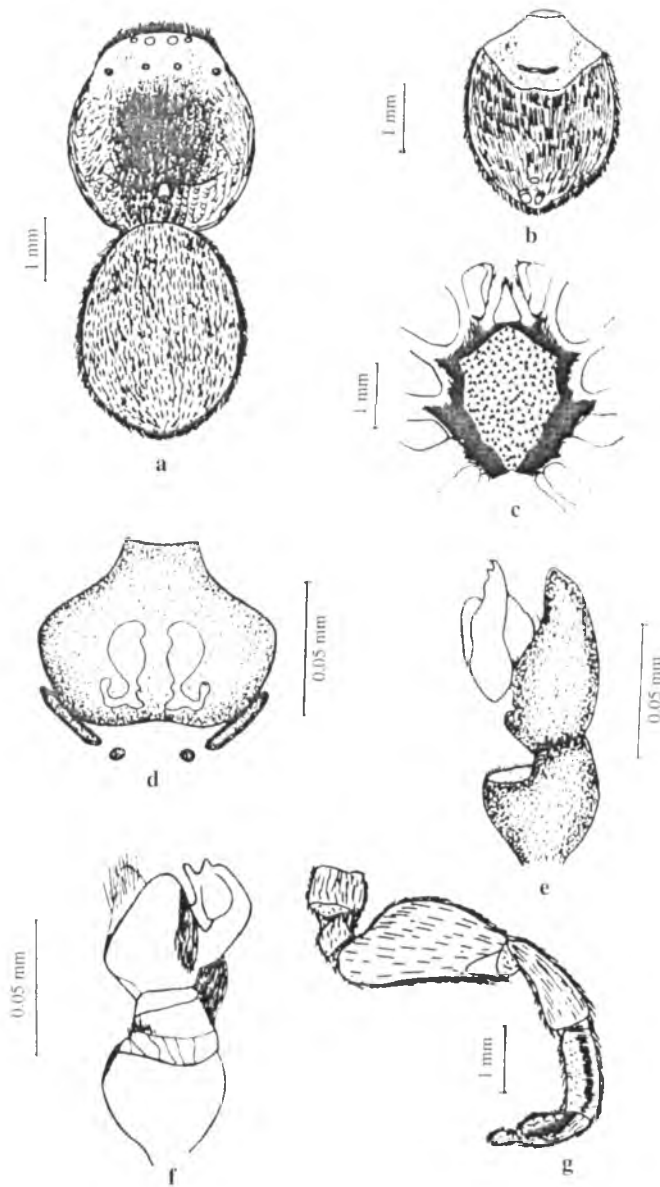
FIGURE 1. *Otiothops namratae*. (a) Dorsal view of female (legs omitted) (b) Ventral view of abdomen showing scutum, (c) Endite and sternum, (d) Internal genitalia, (e) Male palp prolateral view, (f) Male palp retrolateral view, (g) Leg I

Etymology

The species named after my daughter Namrata who helped me in retrieving the specimens from the collection of rare spiders.

Diagnosis

Otiothops namratae sp. nov. is closest to *O. typicus* (Mello-Leitao) in appearance but can be distinguished by the inflated and bifurcated embolus and female genitalia with the spermathecae being widest anteriorly.

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Influence of sugars on growth and development of *Goniozus nephantidis* (Muesbeck), a parasitoid of coconut black headed caterpillar, *Opisina arenosella* Walk

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ABSTRACT: Sugars and honey consumption by parasitoids enhances their growth and development. Effects of individual sugars glucose, fructose, sucrose, trehalose, mixtures of glucose and fructose and 50% honey solution on parasitoid weight gain, egg length, development pattern and survival of *Goniozus nephantidis*, a larval parasitoid of *Opisina arenosella* was assessed. Feeding of parasitoids with sugars and honey enhanced their weight and egg length. The development was better in parasitoids that were fed; 50% honey and glucose + fructose supported better survival. © 2006 Association for Advancement of Entomology

KEYWORDS: *Goniozus nephantidis*, growth and development, adult nutrition

Goniozus nephantidis (Muesbeck) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid, extensively used for the suppression of coconut black headed caterpillar, *Opisina arenosella* (Lepidoptera: Oeocophoridae), a major pest on coconut in India.

Feeding the parasitoids on emergence in the laboratory would improve their mass parasitism in augmentative field releases (Bautista *et al.*, 2001). In laboratory studies it was found that continuous feeding of parasitoid with honey enhanced its longevity and fecundity compared to unfed females (Anonymous, 2005; Subaharan *et al.*, 2005).

The effect of feeding with individual sugars/mixture and honey on adult weight, egg size, and development parameters in *G. nephantidis* was studied. The gut and body sugar level in fed and unfed insects was assessed at intervals.

The study was conducted at Central Plantation Crops Research Institute, Kasaragod. *G. nephantidis* was reared on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) following the standard procedure (Ramadevi *et al.*, 1981).

The newly eclosed mated female parasitoids (12 h old) were placed in a glass vial (2.5 × 10 cm) and fed with concerned sugar solution. The solutions were placed as minute droplets (approx. 10 microlitres) on a wax-coated paper. There were seven

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TABLE 1. Effect of various sugars on development and biological parameters of *G. nephantidis*

Sugar	Body wt. (mg)	Fecundity (No. of eggs laid)	Egg length (μm)	Per cent hatchability	Per cent Adult emergence	Per cent survival (14 days after eclosion)
Sucrose	1.00	10.30	519.35	90.29	80.00	45.00
Glucose	0.97	10.00	530.60	90.00	89.00	51.66
Fructose	0.93	11.26	505.80	94.67	89.34	43.00
Glucose+ Fructose	1.12	11.16	540.12	97.04	93.27	56.66
50%Honey	1.21	11.16	495.51	95.51	92.00	71.66
Trehalose	0.90	9.60	507.83	92.70	88.12	0
Control	0.70	6.67	431.17	55.55	41.3	0
C.D. ($p = 0.05$)	0.02	1.93	25.64	10.06	11.00	3.88

treatments (Table 1). Four replications (10 parasitoids per replication) were maintained for each treatment. *G. nephantidis* females in vials were placed in BOD at $22 \pm 1^{\circ}$C and 12:12: L: D photoperiods. The parasitoids were weighed seven days after feeding and the weight gain was assessed. The egg length of the parasitoids was measured. The fecundity, per cent hatchability, emergence and longevity were observed on 20 parasitoids per treatment.

Twenty *G. nephantidis* females taken in a vial of 2.5×10 cm (three such vials were maintained) were fed once with 50% honey on the day of emergence. Starved parasitoids were provided with water alone. The parasitoids were assayed for the changes in the amounts of glucose, fructose (gut sugars) and trehalose (body sugar) on 2, 4 and 6 days after the commencement of feeding following the method of Olson *et al.* (2000) and Van Handel (1984) based on anthrone reaction.

The results showed that the body weight significantly varied in different treatments (Table 1). The best was 50% honey and it was followed by glucose + fructose, sucrose, glucose, fructose and trehalose. Fecundity in all the treatments were at par and significantly higher than control. Egg length was highest in glucose + fructose treatment and it was also on par with glucose treatment. All the other treatments were at par and significantly inferior to the treatments mentioned above. The shortest eggs were observed in control.

Per cent hatching were at par and significantly higher than in control. Highest adult emergence was observed in glucose + fructose treatment and it was on par with 50% honey. The remaining treatments were at par but were significantly inferior to parasitoids fed with glucose + fructose and 50% honey.

Per cent survival observed on 14 days after emergence showed that 50 per cent of the honey was the best feed and it was followed by glucose + fructose and glucose alone. Sucrose and fructose were at par. There was no survival of parasitoids on 14 days after emergence in trehalose treatment and control.

TABLE 2. Level of sugars in starved and fed *G. nephantidis*

| Sugar | Unfed | | | Fed | | |
| | μg/insect on day | | | μg/insect on day | | |
	2	4	6	2	4	6
Glucose	4.47 ± 0.06	3.06 ± 0.02	2.08 ± 0.08	31.45 ± 0.05	28.96 ± 0.02	20.08 ± 0.01
Fructose	0.52 ± 0.02	0.50 ± 0.02	-0.11 ± 0.01	23.51 ± 0.04	20.20 ± 0.05	13.61 ± 0.01
Trehalose	4.03 ± 0.03	3.12 ± 0.06	2.21 ± 0.01	27.01 ± 0.01	24.98 ± 0.01	22.58 ± 0.02

In the parasitoids that were starved, the gut sugars and body sugars were low as compared to those parasitoids that were fed once on emergence. Though the parasitoids were fed once on emergence there was a rapid decline in both gut sugars and body sugars from day two to day six (Table 2). This decline in the sugar levels stresses the need to increase the frequency of feeding.

Feeding had influence on weight and development parameters of *G. nephantidis*. The reproductive success of an individual female is strongly influenced by its size. Subaharan *et al.* (2005) observed that weight of the female adults influenced the number of mature eggs in the abdomen. Positive correlations between body size and egg load have been observed in *Goniozus ligneri* (O'Neill and Skinners, 1990). Feeding of parasitoids with honey enhanced the fecundity (Anonymous, 2005).

In our studies *G. nephantidis* fed with 50% honey and sugars had larger sized eggs than the unfed parasitoids. Giron and Casas (2003) reported that smaller sized eggs resulted from starved parasitoids.

Parasitoids fed on sugars (except trehalose) and honey exhibited more longevity. The positive intraspecific relationship between body size and longevity of the parasitoids was reported earlier by Godfray (1994). Decline level of sugars may prompt the parasitoid to switch over from host searching to food searching. Wackers (2001) reported that during sugar deprivation in *Cotesia glomerata* it switched over from host searching to food searching.

Feeding the parasitoids on emergence in the mass multiplication centers prior to their release would improve their mass parasitism in augmentative field release as it is evident from the study that the sugar levels of starved parasitoid is low as compared to the fed ones.

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Control of shoot and fruit borer of brinjal, *Leucinodes orbonalis* (Lepidoptera: Pyralidae) in the field

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ABSTRACT: A field study was conducted during 2002–2004 to evaluate Cypermethrin, Carbaryl, Endosulfan, Malathion, Trizophos, *Bacillus thuringiensis* (Bt) and Bt + Carbaryl for the control of brinjal shoot and fruit borer. The results revealed that Cypermethrin (0.007%) and Carbaryl (0.02%) were at par and significantly superior to all other treatments in terms of percent shoot damaged, fruit damage on number and weight basis and on yield basis. © 2006 Association for Advancement of Entomology

KEYWORDS: brinjal, *Leucinodes orbonalis*, ecofriendly insecticides, field evaluation

Brinjal shoot and fruit borer (*Leucinodes orbonalis*) is known to cause about 41 to 72% loss in vegetable crops in Rajasthan (Pareek and Bhargava, 2003). It is thus a limiting factor in successful cultivation of the crop. Many insecticides have been tried against this borer. Several workers reported the effectiveness of synthetic pyrethroids in minimizing the infestation of lepidopterous pests (Mishra, 1993). The incorporation of biopesticides in the pest management programme is gaining importance in recent years (Satpute *et al.*, 2002). Some information on the use of ecofriendly insecticides against this pest is available but not much work has been done in Rajasthan, and hence a two-year field trial was undertaken.

A field experiment was conducted at Umren (Rajasthan) with brinjal cv Pusa purple long in Randomized Block Design (RBD) during 2002–03 and 2003–04 with three replications. The plot size was 3 m × 3 m. There were nine treatments including control (Table 1). Out of four sprays given, first spray was synchronized with the initiation of fruiting and subsequently at fortnightly intervals.

The infestation of shoot borer was recorded on ten tagged plants, which began one week after transplanting seedlings. Fruit borer infestation was assessed separately by observing borer affected and borer free fruits in individual plot at each harvest. Percent fruit borer damage was computed from cumulative data of all the pickings. Data were

TABLE 1. Effect of different insecticides on the incidence of shoot and fruit borer in brinjal during 2002–03 and 2003–04

Treatment	Concentration (%)	Mean percent shoot infestation (%)	Mean percent fruit infestation no. basis (%)	Mean percent fruit infestation weight basis (%)	Yield of healthy fruits (Q/ha)
Endosulfan	0.07	6.27 (14.44)[b]	2.04 (8.12)	11.99 (20.24)[a]	197.45[a]
Malathion	0.05	8.61 (16.99)[c]	7.69 (16.04)	17.69 (24.86)[c]	152.48
Carbaryl	0.20	4.30 (11.91)[a]	3.46 (10.61)[a]	9.94 (18.36)[a]	205.75[a]
Bacillus thuringiensis (Bt)	0.012	10.74 (19.06)	10.54 (18.90)	18.24 (25.28)[c]	136.17
Bt + Endosulfan	0.012 + 0.03	6.72 (14.96)[b]	3.58 (10.85)[a]	15.09 (22.82)[b]	168.37[b]
Bt + Carbaryl	0.012 + 0.10	6.34 (14.52)[b]	4.35 (12.03)[a]	14.08 (22.01)[b]	181.21[b]
Cypermethrin	0.07	3.92 (11.74)[a]	1.24 (6.28)	7.31 (15.66)[c]	220.46
Trizophos	0.10	8.74 (17.11)[c]	7.12 (15.43)	17.11 (24.39)	153.56
Control	–	17.31 (24.55)	17.86 (24.99)	38.23 (38.2)	92.57
C.D at 5%	1.38	0.85	2.73		

Figures in parentheses are angular transformed values. Figures with the same letter are on par at $p = 0.05$.

subjected to analysis of variance. Healthy fruits of all harvest in each plot were pooled together to work out the total yield.

The data in Table 1 revealed that all the treatments were superior to control in reducing shoot and fruit borer incidence. Cypermethrin (0.007%) and Carbaryl (0.20%) recorded significantly lowest shoot infestation (3.92 and 4.30%). Endosulfan, Bt + Endosulfan were statistically at par in efficacy with shoot infestation ranging from 6.27 to 6.72%. Bt alone (0.012%) ranked as least effective treatment with shoot damage of 10.74 percent. George *et al.* (2002) reported the effectiveness of synthetic pyrethroids and recommended them along with Carbaryl and Endosulfan.

Cypermethrin proved to be significantly superior to all other treatments having 1.24% fruit infestation on number basis followed by Endosulfan 2.04%. Duara *et al.* (2003) also reported that the number of fruits were higher in pyrethroid treated plot. Cypermethrin with 7.31% fruit damage gave significantly better protection than other insecticides on weight basis. Carbaryl and Endosulfan forms the second best group of treatments with fruit infestation of 9.94 and 11.99% respectively. Bt + Carbaryl and Bt + Endosulfan were on par with infestation of 14.08 and 15.09%, respectively. Bt alone was least effective (18.24%). Singh and Singh (2003) our results reported that Cypermethrin was highly effective against pest and resulted in higher yield compared to conventional insecticides. The efficacy of chlorinated hydrocarbons Carbaryl and Endosulfan were also reported earlier by Roy and Panda (1994).

ACKNOWLEDGEMENTS

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Foraging behavior of *Apis mellifera* L. (Hymenoptera: Apidae) on *Brassica juncea*

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ABSTRACT: Foraging behavior of *Apis mellifera* was studied on oil seed crop *Brassica juncea*. Maximum foraging activity was recorded during noon times which is very positively correlated with the day temperature. Also weight of pollen load carried, time spent on one flower and number of flowers visited were also maximum at the 1200 hours. © 2006 Association for Advancement of Entomology

KEYWORDS: Foraging, *Apis Mellifera*, *Brassica juncea*

INTRODUCTION

Foraging behavior of *A. mellifera* has been recorded by different workers on different crops in different localities (Gupta *et al.*, 1984; Verma and Dutta, 1986; Kapoor and Dhaliwal, 1989). A survey of literature, however, shows that such studies have not been carried out on *B. juncea*, which is very widely cultivated in different parts of Northern India. Hence a brief study was undertaken during Oct./Nov.1996.

MATERIAL AND METHODS

The present work has been conducted during flowering season of *Brassica juncea* (Mid September to mid of November) at Togan village (Punjab). The collected data were statistically analysed: to estimate standard deviation, co-efficient of correlation with weather factor.

OBSERVATIONS AND DISCUSSIONS

Time of commencement and cessation of flight activity was observed in case of *A. mellifera*. Bees start foraging on *B. juncea* at 0611 hours and stops foraging at 1742 hours, spending a total of 1138 hours in the field. Time of commencement of

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foraging activity was poorly correlated with temperature but time of cessation was strongly correlated with temperature as it showed backward shift (Table 1). Many workers have studied the time of commencement and cessation of flight activity of this species on different crops and fruits tree and reported more than ten hours of flight period on *Plectranth rogosus* (Gupta *et al.*, 1984), apple (Verma and Dutta, 1986), cauliflower (Kapoor and Dhaliwal, 1989), cherry (Kumar, 1990) and almond (Kumar, 1995) and less than nine hours in *Gossypium* and cauliflower (Tanda and Goyal, 1979) and Kapoor and Dhaliwal (1989) respectively. Rinderer *et al.* (1985) reported that every colony of African bees began to fly in large number at sun rise and last bee returned to respective colony after sun set. It appears that the starting of flight, stoppage of flight and total hours of activity varies not only in different months of year but also depends on the crop they forage. Earlier studies by Parker, 1926 on the foraging behavior of *Apis mellifera* in Ithaca, New York showed peak pollen collection between 0800 to 0900 hours, declines towards mid day and secondary peak between 1400 to 1500 hours. Gupta *et al.* (1984) reported that the number of worker bees forage on flowers increased until 1400 hours and declined thereafter until 1700 hours. Kapoor and Dhaliwal (1989) observed that number of pollen foragers of *Apis mellifera* decreased during 0900 to 1000 hours, but showed variation. Raj and Rana (1993a) the foraging activity is highest at 1200 as compared to 0900 and 1500 hours. Kumar (1995) reported that flight activity increase smoothly from 0800 to 1300 hours and then showed a constant decreases up to 1700 hours. The present observations showed the maximum activity of worker bees was at 1200 noon.The present finding was supported by the works of Kefuss and Nye (1970), Gupta *et al.* (1984); Verma and Dutta (1986); Raj and Rana (1993a). However, Bonnier (1878) and Ben-Nerya (1937), Gray and Witherall (1977) noted that the variation is related to different factors such as availability of food, environmental condition & foraging localities.

The earlier reports on the actual weight of pollen carried by *A. meliffera* showed that the 12.22 mg pollen of apple (Verma and Dutta, 1986), 12.15 mg of *Plectranthus rogosus* (Sharma 1989), 15.50 mg of plum (Rana, 1989) and 13.28 mg pollen of *Brassica compestris* (Raj *et al.*, 1993b). In the present observation it was reported that maximum pollen load carried by worker bee was 11.20 mg at 1200 noon. However, some workers have observed that weight actually depends on the plant species foraged (Park, 1922). While other feels that the weight depends on the relative size of body parts of the species concerned (Karmo and Vickey, 1954; Kendell and Solomon, 1973; Mattu and Verma, 1980). There are several reports on time spent by *Apis mellifera* on crops and fruits other than *Brassica juncea*. Rymahesvskii (1956) has reported more then 30 seconds on the flowers of apple, apricot, respberry and black current; Kumar (1995) less than 21 seconds in almond and peach; Verma and Dutta (1986) 5.29 seconds and 6.65 seconds on the flowers of cherry and apple respectively. It is thus evident that in case of *Apis mellifera* there is wide variation in the time spent on each flower of different fruits trees. In the present report it was noticed 2.44 seconds at 1500 hours on *Brassica juncea*. In the other hand the time spent by *Apis carana indica* and *Apis florea* on the flowers of *Brassica compestis* have been reported 3 second and

TABLE 1. Foraging behavior of *Apis mellifera* on *Brassica juncea* at Togan village (Punjab)

Time	0900	1200	1500	1700
Mean time of commencement /Mean time of cessation of flight activity	0611 (0.26)*	---	---	1749 (1)*
No. of bees leaving the hive	28.2 ± 12.19	46.0 ± 6.11	27.91 ± 10.73	9.9 ± 3.85
No. bees entering the hive	15.3 ± 5.9	20.7 ± 3.46	12.5 ± 2.84	2.20 ± 0.87
Pollen load carried by one worker bee	7.35 ± 2.10	11.20 ± 0.85	6.40 ± 1.57	N.A
No. of *Brassica* flower visited by each bee/minute	17.06 ± 1.48	25.05 ± 1.40	21.75 ± 0.79	N.A
Time spent (second) by worker in one flower of *Brassica juncea*	2.28 ± 0.1	1.74 ± 0.1	2.44 ± 0.1	N.A.

*Coefficient of correlation.

6.45 second respectively by Murrell and Williams (1981). The difference in the time spent on the flowers of fruits crops may be dependence on many factors such as the size of flower and also the amount of viscosity of the nectar present in the flowers.

Although much work has been done on the number of flowers of different fruits and crops visited by *A. meliffera* and other species of *Apis*, Tanda (1983) reported that the workers bee of this species visited 5 to 7 flower of *Gossypium arboruim L* per minute; Verma and Dutta (1986) 3.33 flowers of apple; Kumar (1995) 4.59 and 3.74 flowers of almond and peach respectively. However, in the present study it was observed that the number of flowers visited was appreciably more at noon time as compared to morning and evening hours and visited 25.05 flowers of *Brassica juncea* at 1200 hours.The present results was in conformity to Gupta *et al.* (1984) who stated that worker bees visited 25.8 and 33.6 flowers per minutes in *Plectranthus rogosus* during same hours.

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